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 MUSCULATURE WITH PARTICULAR REFERENCE TO
 ADOLESCENT IDIOPATHIC SCOLIOSIS
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HUMAN AND MONKEY PARAVERTEBRAL MUSCULATURE WITH PARTICULAR
REFERENCE TO ADOLESCENT IDIOPATHIC SCOLIOSIS

by



DONNA MARIE FORD

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
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ANATOMY

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THE UNIVERSITY OF ALBERTA
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled HUMAN AND MONKEY PARAVERTEBRAL MUSCULATURE WITH PARTICULAR REFERENCE TO ADOLESCENT IDIOPATHIC SCOLIOSIS submitted by DONNA MARIE FORD in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

ABSTRACT

The major aim of the present project was to obtain "normal" values for muscle fiber characteristics of proportion, size and strength factor in human paravertebral musculature to determine differences, if any, in characteristics associated with patients with idiopathic scoliosis, and to establish, relate and compare these characteristics with those found at specific vertebral levels in the rhesus monkey for comparison as an animal model for the study of scoliosis.

Biopsies of sacrospinalis and multifidus muscles were taken from both sides of the vertebral column adjacent to lumbar vertebra 5, from nineteen patients aged 28 to 73 years (12 males and 7 females: mean age 45.3 years), suffering from acute lumbar disc protrusion. The samples used were from subjects who had a relatively brief history of spinal dysfunction. The respective muscle fiber morphology and characteristics were assessed using Trichrome, NADH and Adenosine Triphosphatase (ATPase) staining procedures on transverse sections of portions of whole muscles. A "strength factor" component was developed for each muscle by combining measurements of fiber area and fiber type, to introduce a functional indication of potential force. While a large difference in muscle fiber characteristics may exist on opposite sides of the vertebral column these differences do not appear to be related to the

side of the disc protrusion or age or gender of the individual. Therefore it is suggested that these standards be used as representative of normal muscle in comparative studies involving abnormal paravertebral muscle.

In addition, twelve paravertebral muscle biopsy samples were taken at operation for spinal instrumentation from each of seven patients suffering from idiopathic scoliosis (1 male and 6 females: mean age 14.3 years). The samples were collected from two specific sites (superficial: longissimus thoracis; deep: multifidus) on both sides of the vertebral column at the level of the apex of the primary curve (Thoracic 8 to 11) and two vertebral levels above and below the apex. The results of this study support and extend the findings of other workers. Not only was a significantly larger percentage of Type I fibers found in multifidus muscle at the apex of the curve on the convex side as other workers have found but also in the superficial muscles above and below the apex of the curve on the convex side. These results present a complex picture of muscle fiber characteristics associated with idiopathic scoliosis.

With the intention of creating an animal model for the study of experimentally induced scoliosis and subsequent comparison with human paravertebral muscle, twelve biopsy samples of superficial and deep paravertebral muscle were taken at various sites (thoracic 3 and 8; lumbar 3) from each of 5 adult (2-3 years) female rhesus monkeys within 2 hours of sacrifice. A significant decrease in percentage of

type I muscle fibers existed with descent of the column on both sides in superficial and deep layers (with a concomitant increase in percentage of type II fibers). A similar pattern existed with regards to muscle fiber size and may reflect differences in function of the vertebral muscles in quadrupeds.

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I. INTRODUCTION

The identification of skeletal muscle fiber types by histological and histochemical techniques has been used extensively in the past as a method of assessing the constituents and state of health of muscle groups both in humans and animals. These muscle fiber types have been classified in both anatomical and physiological terms. By localizing the enzyme systems and other chemical constituents at a cellular level, these findings can be subsequently related to the functional significance of the muscle fibers and group.

Due to legal and ethical problems governing the availability of human tissue, large comprehensive studies of the so-called "normal" individual are lacking. Researchers have turned to available muscle acquired at operation for various reasons or animal tissue, attempting to associate their findings with normal human tissue. As a result, the amount of information available on human paravertebral muscle is limited. It appears necessary therefore to determine and characterize as near as possible, what is or can be accepted as normal paravertebral muscle and compare these characteristics with suspected abnormal paravertebral muscle such as in idiopathic scoliosis.

Idiopathic scoliosis (IS) is the commonest form of scoliosis (75-80%; Harrington, 1977) occurring during the sensitive years in growing adolescent females and its

effects can be extremely devastating. The features of idiopathic scoliosis are lateral deviation of the vertebral column usually with vertebral rotation in which the spinous processes are directed toward the side of the concavity, and with an accompanying rib hump on the side of the convexity. Basic knowledge of the etiology, pathogenesis and biomechanics of scoliosis is at an early stage although muscle has been implicated (Fidler *et al.* 1974; Fidler and Jowett, 1976; Spencer and Eccles, 1976; Spencer and Zorab, 1976; Yarom and Robin, 1979, 1979b; Green, 1981). Studies dealing specifically with the the histological and histochemical analysis of paravertebral muscle in idiopathic scoliosis have been performed and these suggest the presence of abnormalities in muscle fibers and an imbalance in fiber type proportions at the site of the major curvature. Referral will be made to the almost insuperable problems associated with idiopathic scoliosis and those structures intimately concerned with the vertebral column. However, due to the limitations and priorities of this study, selected histological and histochemical properties of paravertebral muscle will be emphasized.

The primary purpose of the present study was therefore:

1. To obtain "normal" standards for human paravertebral muscle fiber characteristics at a specific level in the lumbar region of the vertebral column. Confirmation of the assumption that individuals exhibiting a straight spine (although suffering disc dysfunction) also possess muscle

fiber characteristics unaffected by the disc protrusion would enable comparison of these standards with those muscle fiber characteristics of abnormal paravertebral muscle. Re-evaluation of human paravertebral musculature at one vertebral level gives vital information preliminary to future studies involving analysis of paravertebral muscle at various levels of the vertebral column from human autopsy and trauma subjects.

2. To examine samples taken at operation for spinal instrumentation from patients with idiopathic scoliosis to determine differences, if any, in muscle fiber characteristics associated with the scoliosis. Subsequent comparison of these characteristics with those of muscle taken from subjects suffering spinal disorders (i.e. patients suffering disc dysfunction) might further enhance our knowledge of the etiology and/or progression of scoliosis.

3. To establish, relate and compare paravertebral muscle fiber characteristics found at specific vertebral levels in the rhesus monkey with those of the human. Significant similarities in these muscle fiber characteristics would be inferential to the use of the rhesus monkey as an appropriate animal model for the study of scoliosis.

A. SKELETAL MUSCLE DEVELOPMENT, COMPOSITION AND INNERVATION

Skeletal muscle cells are derived from mesenchymal cells. Upon elaboration of the notochord and neural tube in the human embryo, the mesoderm to the side of these structures thickens to form two longitudinal columns, the paraxial mesoderm (Fischman, 1972; Moore, 1983). The paraxial mesoderm gives rise to the somites which begin forming at about the beginning of the fourth week or nineteenth day (Streeter, 1945; O'Rahilly, 1979). The somites appear as a series of cuboidal or square elevations along the dorsolateral surface of the embryo; eventually 42-44 pairs develop during the so-called "somite period" over a period of about 10 days. In section, a small slit-like lumen, the myocoele, is said to occur in the center of each wedge-shaped somite and this increases in size until the somite appears as a hollow vesicle with thick walls. The wall differentiates into a dorsolateral part, the dermomyotome and a ventromedial part, the sclerotome. The dermomyotome contributes to the integument and voluntary muscles of the trunk, neck and part of the head; the sclerotome participates in the development of the axial skeleton (base of skull, vertebrae, sternum, ribs and costal cartilages).

Each subsequent myotome divides into dorsal (epaxial) and ventral (hypaxial) regions. The dorsal division gives rise to the vertebral muscles, innervated by the dorsal ramus of a corresponding spinal nerve and the ventral

division migrates ventrally in the body wall or somatopleure to become the anterior axial muscles, innervated by the corresponding ventral ramus.

Gray (1973) states that except for particular muscles of the head and neck, and the limb muscles, all somatic muscles (striated and voluntary) are derived from the myotomes. There is little doubt that the limb muscles develop in situ from mesenchyme derived from the lateral plate mesoderm (somatopleure) (Fischman, 1972). However, a point of controversy appears to exist regarding the ventral two-thirds of the trunk. Straus and Rawles (1953) concluded that lateral plate rather than myotome is the source for at least the ventral half of the trunk musculature whereas (Detwiler, 1955) determined the source as being of somitic origin. This disagreement appears to be unresolved at this time as Langman (1981) states that the ventrolateral body wall originates from the somitic mesoderm. Agreement exists at least between Straus and Rawles (1953), Gray (1973) and Langman (1981) that paravertebral muscles are of somitic origin.

In the differentiation process of mesenchymal tissue into muscle cells, the primitive cells can be classified as they mature and progress through a series of stages (Fischman, 1972; Schloen *et al.* 1979; Minguetti and Mair, 1981). The accepted mammalian myogenic process or stages of skeletal muscle development are as follows and the nomenclature used is that of Boyd (1960).

1. PREMYOBLAST: undistinguishable mesenchyme.
2. PRESUMPTIVE MYOBLAST: syncitium of bipolar cells incapable of fusion or synthesis of myofibrils.
3. MYOBLAST: spindle shape, post-mitotic mononucleated cells capable of fusion and synthesis of contractile proteins.
4. MYOTUBE: elongated cells with centrally placed nuclei; at times horseshoe shaped; loosely organized bundles which may display the beginnings of fascicles; peripherally disposed myofilament formation in the sarcoplasm identifiable with myosin adenosine triphosphatase (ATPase).
5. MYOFIBER: cylindrical long fibers polygonal in shape with peripherally placed nuclei; made up mostly of myofilament clusters; obvious merger of bundles of myofibers into fasciculi.

The eventual muscle cell or myofiber has a circular, elliptical or polygonal profile in cross-section, is 10-100 μ m in diameter in adult human muscles and may achieve a length of over 30cm in long muscle (Gray, 1973). Many flattened peripheral nuclei lie deep to the cell membrane or sarcolemma while the cytoplasm of the muscle cell, the sarcoplasm, contains "hundreds to thousands" (Dubowitz and Brooke, 1973) of myofibrils. Myofibers are surrounded by a connective tissue covering known as endomysium; groups of fibers in bundles or fasciculi are surrounded by a stronger connective tissue sheath known as perimysium; and finally groups of fasciculi surrounded by a rather strong connective

tissue sheath, the epimysium, form the muscle proper.

With various investigative techniques these muscle fibers have been found to possess different characteristics by which they can be classified into two major fiber types (type I and II) according to their enzyme and chemical constituents and ultimate functional activity. In man, the muscles are mixed (heterogeneous) with regard to muscle fiber composition and the different fiber types give the muscle a mosaic or checkerboard appearance (Dubowitz and Brooke, 1973; Saltin *et al.* 1977).

Dubowitz (1963, 1965) investigated the enzymatic maturation of muscle fibers in man, guinea pig, hamster, rabbit and rat. Except in the rabbit and rat (in which differentiation started 2-3 days postnatally and concluded at about 14 days) all other subjects showed complete maturation and differentiation into muscle fiber types at birth. Studies by Wirsen and Larson (1964) and Buller *et al.* (1960) of the mouse and cat respectively also demonstrated an immature fiber population at birth.

Beatty (1966, 1967) investigated a number of limb muscles in 11 different species of adult monkeys and concluded that most primate muscles consist of varying ratios of both types of muscle fibers. It was demonstrated that differentiation was complete in monkeys at 120 days gestation and that muscle fibers at 150 days gestation were equivalent to those of a 3 day old human infant. Furthermore, differentiation at 73% (120 days to term) of

the gestation period in monkeys was equivalent to 75% gestation period in the human, with an eventual adult body weight in both species being comprised of 40% muscle.

Van Wageningen and Catchpole (1965) concluded that although man matures later than monkeys, the rhesus monkey has similar points of timing and is ideally suited to the study of many phases of growth and development as a paradigm of man. They cautioned that existing data are limited because in the research lab it is rare to find "uninterfered with" normal monkeys of known age. Based on a sample of 225 monkeys, they determined the gestation period to be between 162 and 177 days (mean 168.9 ± 6.85 days).

Interest has been shown in the satellite cell (myosatellite cell) a mononucleated cell lying beneath the basal lamina of an adjacent myofiber in the mature muscle (Ontell, 1977; Nag and Foster, 1981). Salleo *et al.* (1980) regard satellite cells as dormant myoblasts which are extremely numerous during the first 2 weeks of postnatal life but which diminish with age. Such cells, according to an extensive summary of the literature by Fischman (1972) appear to be a possible source of myogenic cells during muscle regeneration following muscle trauma. It was noted that these cells continue to divide in early postnatal life of birds and animals and act as a source for new muscle nuclei during rapid growth of muscle fibers (Moss and Leblond, 1971). Using electron microscopy (EM), Salleo *et al.* (1980) reported that during compensatory hypertrophy

satellite cells were observed to change by initially enlarging, becoming free of the connective tissue sheath network, proliferating into rows of cells, finally forming elongated structures which ultimately developed new muscle fibers. They further determined that satellite cells proliferate during aging into daughter cells which are then incorporated into mature fibers, subsequently amounting to only 4-8% of the total number of nuclei in muscles of adult animals. Also using EM, Minguetti and Mair (1981) suggested that satellite cells evolve from a separate stem of myoblasts, reaching the myotube stage and remaining under the plasma membrane of a more mature muscle fiber until some stimulus in subsequent life requires it to continue development. Hoyle (1983) on the other hand reported that different muscle cell types originate from a single cell lineage, with the potential of becoming any type or subcategory.

Satellite cells have been a subject of contemplation in IS as an imbalance in fiber type proportion and size at the site of the major curvature has been found (Fidler *et al.* 1974; Fidler and Jowett, 1976; Spencer and Eccles, 1976; Spencer and Zorab, 1976; Yarom and Robin, 1979, 1979b; Green, 1981; Ford *et al.* 1983). If satellite cells are dormant myoblasts (Salleo *et al.* 1980) capable of differentiating into mature muscle fibers of required type, then an imbalance in fiber type would be an accepted phenomenon. An attempt by these cells to compensate for the

"bending forces" in IS may constitute an appropriate stimulus for continued development. On the other hand, Stickland (1981) reported that muscle fiber number appears to be genetically determined and that the number of muscle fibers remains constant after birth (Buchthal and Schmalbruch, 1980).

Hoyle (1983) suggested that while hypertrophy results in an increase in muscle fiber size and the amount of collagen and connective tissue, it is unlikely that new cells are added resulting in a change of fiber number (hyperplasia). It has also been suggested that hyperplasia may be produced by fiber splitting, accounting for an imbalance in fiber proportion (Gollnick *et al.* 1980). This latter hypothesis has been supported by the findings of Ho *et al.* (1977) who suggested that muscle fiber splitting may represent a physiological adaptation of muscle to overload. Minguetti and Mair (1981) found no evidence of "splitting" of the differentiated muscle fibers to produce new muscle fibers and suggested that the use of light microscopy (LM) (as for Ho *et al.* 1977) did not permit accurate visualization of appropriate cell membranes.

The primary function of skeletal muscle and individual muscle fibers is that of contraction and relaxation, performed by an interaction of contractile proteins actin and myosin under the influence of electrical impulses. Individual muscle fibers within each muscle are arranged in such a manner as to develop a force between the two ends of

the muscle when stimulated. Microscopically, striated muscle fibers show alternate light bands (I bands) and dark bands (A bands) along their length. Each muscle fiber is made up of longitudinally disposed fine myofilaments and displays highly regular light and dark bands stacked in register. At the center of the A band is a lighter zone, the H band (with an intersecting M line) and at the center of the I band is a slightly darker area known as the Z line. The region between the Z lines is the contractile unit or sarcomere and when a muscle contracts the two Z lines move closer together. A sarcomere consists of a series of overlapping thick (myosin) and thin (actin) filaments (Huxley, 1965; Leeson and Leeson, 1981; Hoyle, 1983). During longitudinal growth of striated muscle cells, new sarcomeres of nearly constant length are formed at their ends well in line with those of neighboring muscle cells (Hoyle, 1983). However, it appears that mismatching is frequent during later lateral additions and elongations and most common in phasic, type II muscle fibers. The myosin filaments are relatively fixed and restricted to the A band with projections (crossbridges) which interact in a cyclic attachment and re-attachment activity to the actin filaments during muscle contraction.

The intermyofibrillar space contains aqueous sarcoplasm in which the various organelles are found: mitochondria; glycogen granules; sarcoplasmic reticulum and transverse tubular system.

a. MITOCHONDRIA: Are observed most frequently on either side of the Z line and immediately adjoining the A band and are concerned with the energy supply of the muscle fiber. An abundance of mitochondria appears to be related to the resistance of a muscle to fatigue (Dubowitz and Brooke, 1973) determining its oxidative capacity, tonic type I fibers having more (Ogata, 1958, 1964).

b. GLYCOGEN GRANULES: Situated in the sarcoplasmic space and although they tend to occur mostly in the I band zone, they are not limited to any one part of the muscle fiber. Glycogen granules provide a means of rapid energy.

c. SARCOPLASMIC RETICULUM AND TRANSVERSE TUBULAR SYSTEM: The sarcoplasmic reticulum (SR) is a regular repeating structure of longitudinally oriented tubules filled with amorphous material which surround the myofibrils and which coalesce at the A-I junction into terminal cisternae. The transverse tubular system (TT), an invagination of sarcolemma in a transverse direction, penetrates amongst the myofibrils and arborizes in a transverse plane between the A and I bands. It is not continuous with but in intimate contact with two terminal cisternae of the SR and together they represent a muscle triad. Each sarcomere then is in association with two muscle triads. In muscle contraction, the action potential generated at the neuromuscular junction on the sarcolemma is propagated along the muscle fiber membrane and into the muscle fiber along the TT system. Depolarization of the

T-tubule membrane causes release of calcium ions from the terminal cisternae which then initiates muscle contraction. Adenosine triphosphate (ATP) hydrolysis can be directly related to specific phases of the crossbridge cycle (Fuchs, 1974).

The two independent systems of fine tubes, the SR and the TT system, provide a means of communication between the nerve impulse and the myofibrils (Bennett and Porter, 1953; Hoyle, 1983). It is now generally accepted (Leeson and Leeson, 1981; Hoyle, 1983) that the actin filaments attached together at the Z line, slide into the spaces between the myosin filaments when a muscle contracts (sliding filament theory) with no change in length of either filament. As stated previously and referenced extensively (Huxley, 1965; Fuchs, 1974; Landau, 1980; Hoyle, 1983) muscle contraction is a result of a change in site of attachment of the crossbridges of the myosin filament to the actin filament occurring in a successive manner of attachment, detachment and re-attachment.

The supply of energy for contraction and relaxation is provided by the breakdown of the stored ATP and glycogen. ATP is the primary or first hand energy provider for muscle contraction. During muscle contraction ATP breaks down to adenosine diphosphate (ADP), losing one phosphate molecule. Creatine phosphate (CP) is available to resynthesize ATP from ADP ($\text{ADP} + \text{CP} = \text{Creatine} + \text{ATP}$), an activity which can go on theoretically until the supply of creatine phosphate

is exhausted. Additionally, the breakdown of muscle glycogen or glucose into pyruvic acid provides high energy phosphates which are available to change ADP back into ATP. Pyruvic acid is then changed to lactic acid by picking up one hydrogen ion. An accumulation of lactic acid along with exhaustion of the supply of oxygen becomes the main limiting factor in muscular activity and causes fatigue. The muscle also builds up an oxygen debt that must eventually be repaid. These two sources of ATP are limiting insofar as the length of time they can operate and are readily exhausted in activities requiring all-out effort such as sprinting. These physiological changes occur in the absence of oxygen and are referred to as the anaerobic system of energy. A third source of ATP for muscle contraction comes from aerobic activity in which the effort is less intense and can be continued for an extended period of time. The oxygen supply to the muscle is sufficient to oxidize and resynthesize the lactic acid into glycogen with the release of carbon dioxide, water and energy. The availability of oxygen to the muscle cells in the final analysis is what determines endurance in prolonged physical work. There is minimal or no lactic acid accumulation during aerobic work and also no oxygen debt. When the body works at a level at which there is no oxygen debt build-up, it is said to be in a "steady state".

Muscle fibers which are expected to maintain tone or a state of constant force over a long period of time (tonic,

type I) require a continuous supply of oxygen and of substrate mediated by the capillaries and to a large extent metabolized by the enzymes of the mitochondria. Blood flow through a muscle composed mainly of these fibers is maintained during contraction ensuring an adequate supply of oxygen which determines endurance (Buchthal and Schmalbruch, 1980). Alternately, a muscle containing more fibers which are capable of producing rapid intermittent bursts of activity (phasic, type II) readily restricts the blood flow through the muscle forcing the fibers to switch to anaerobic metabolism, rapidly depleting their energy stores.

Histochemistry enables one to visualize the differences in the content of metabolic enzymes; tonic fibers are rich in oxidative and poor in glycolytic enzymes whereas phasic fibers are rich in glycolytic and poor in oxidative enzymes (Buchthal and Schmalbruch, 1980). Therefore, some fibers (type I) possess highly oxidative metabolism while others (type II) are predominantly anaerobic (Dubowitz and Pearse, 1960).

In movement, the basic component of motor activity is the motor unit (MU) consisting of an anterior horn cell, its axon and the skeletal muscle fibers which are innervated by that axon (Buchthal and Schmalbruch, 1980). Mammalian skeletal muscle generally contains at least two types of muscle fibers, revealed by histochemical techniques notably ATPase (Burke and Tsairis, 1974; Gutmann and Syrový, 1977; Maton, 1980). Because contraction occurs by the interaction

of actin, myosin and ATP and because the speed of contraction has been shown to be directly proportional to the muscle's myosin activity in the adult (Barany, 1967) muscles can be categorized as fast-twitch (type II; also further divided into type IIA or IIB fibers) and slow-twitch (type I) by histochemical means as can their motor units (Maton, 1980). Motor units were reported as being basically of two types (small and slow: type I fiber; large and fast: type II fiber) (Maton, 1980) and as being enzymatically different (Yellin and Guth, 1970), determining the mechanical and biochemical properties of a given muscle fiber. It has also been established that the speed of muscle contraction is neurally regulated as cross-innervation studies demonstrated a reversal of contraction time or twitch characteristics and myosin ATPase activity (Buller *et al.* 1960; Dubowitz, 1967). Intermittent long-term stimulation additionally showed transformation of a slow to a fast-twitch muscle (Pette *et al.* 1975), the changes involving mechanical properties, enzymatic activity and the properties of myosin (Pette *et al.* 1975; Buchthal and Schmalbruch, 1980). However, Gollnick *et al.* (1980) state that type IIA and IIB fibers appear to be identical in their basic characteristics and although interconversion by endurance training may occur (i.e. type IIA's become type IIB's) the meaning of this is not clear. Slow-twitch units which reportedly have greater blood supply and oxidative metabolic sources are much less fatigueable than fast twitch

(Guth and Yellin, 1971; Wolf, 1980). Furthermore, the muscle fibers of a particular motor unit are homogeneous (of the same histochemical type) and presumably have the same physiological properties (Edstrom & Kugelberg, 1968; Kugelberg, 1976; Gollnick, 1980).

The fibers of many MU's intermingle in a given territory, are more concentrated near the center of the muscle but not necessarily in the same fasciculus, and are not ordinarily in contact with other fibers of the same MU (Buchthal and Schmalbruch, 1980). The number of muscle fibers included in a single MU varies within and between muscles. Where precise control is required, fewer muscle fibers are included in a given MU (e.g. ocular and intrinsic hand muscles).

It is generally agreed (Maton, 1980; Buchthal and Schmalbruch, 1980) that when increasingly heavier loads are applied during isometric and isotonic muscle contraction there is a progressive recruitment of MU's according to the size principle (Henneman and Olsen, 1965) of slow to fast MU's. Different MU's are recruited in accordance with the force, not the type of contraction. Therefore the expressions "tonic" and "phasic" contractions are pertinent to MU twitches but not necessarily to specific voluntary function (Maton, 1980). Type I fibers can develop small tensions at a slow rate (long time to peak tension) whereas type II fibers can produce the largest tetanic tension at a rapid rate (short time to peak tension) (Edgerton, 1976). A

classification system based on physiological and histochemical observations in one species or for MU's in one muscle is not necessarily applicable to another muscle within the same species or the MU's of another species. Also, some mammals do not exhibit full differentiation of fiber types at birth (Buller *et al.* 1960 Close, 1964; Goldspink, 1972; Gutmann and Syrový, 1977) and MU's may be uniformly slow at birth (Denny-Brown, 1929; Dubowitz and Brooke, 1973) although with high ATPase activity.

B. MOVEMENTS AND MUSCLES OF THE VERTEBRAL COLUMN

The vertebral column may be considered as a segmented longitudinal structure composed of a series of bony units (vertebrae) firmly connected to one another by joints and ligaments, surrounded by periarticular musculature and affording static support and kinetic function.

The smallest working component of the column, designated by Panjabi *et al.* (1981) as a Functional Spinal Unit (FSU) consists of two adjacent vertebrae and their intervening soft tissue. Each FSU has been further divided into 2 portions consisting of anterior and posterior components:

Anterior: Vertebral body; Intervertebral disc;
Anterior and posterior longitudinal
ligaments.

Posterior: Neural arch; Inter and Supraspinous ligaments;
Ligamentum flavum; Intertransverse ligaments;

Articular capsules of apophyseal joints.

White (1971) further classifies the posterior vertebral component as a "posterior element", consisting of facet joints (apophyseal), laminae, ligaments and spinous processes. The sum total of all the FSU's superimposed one upon the other maintains a balance against gravity and a flexibility for the structures providing movement. It is uniquely designed for support and movement of the trunk as well as support and stabilization of the upper limb and head (Gray, 1973; Hollinshead and Jenkins, 1981).

It is well documented and accepted (Anson, 1966; Gray, 1973; Basmajian, 1982; and O'Rahilly, 1983; Woodburne, 1983) that the vertebral column when viewed in profile possesses four curvatures situated in the cervical, thoracic, lumbar and sacro-coccygeal regions. Of these, the thoracic and pelvic curves are considered to be primary, having commenced development in utero, and have their concavities directed ventrally. The cervical and lumbar curves on the other hand, have their convexities directed ventrally and are usually considered as secondary or compensatory curves, developing postnatally as a means of maintaining balance in the upright posture. However, a study (Bagnall *et al.* 1977) involving the radiographic examination of 156 fetuses, determined that in fact 83% of their cases already possessed a secondary cervical curvature as early as 9 1/2 weeks conceptual age.

Of particular interest is the reference made (Jackson, 1914; Anson, 1966; Gray, 1973) to a fifth curve, being normally present and of lateral disposition in the thoracic region, a point virtually ignored in most reference descriptions. Moe *et al.* (1978) stated that a lateral deviation does not exist in the normal spine when viewed from the anterior and posterior sides although their methods of assessment were not clarified. Idiopathic scoliosis exhibits a lateral deviation of the vertebral column which coincidentally has as its major site of deviation, the thoracic region. The pathological lateral deviation in IS can be of varying degrees of curvature and when seen clinically can be well outside the limits of normality. Jackson (1914) reported that in most cases the curve is directed to the right in the upper thoracic region and suggested that although race and occupation could cause modification of the curve, the greater use of the right hand is probably a contributing factor to its deviation. Anson (1966) on the other hand cited a study (Miles, 1944) in which 10 out of 70 undisturbed columns demonstrated a marked lateral thoracic curvature, equally distributed to both left and right sides. Additionally, the heights of the vertebral bodies and intervertebral foramina were found to be greater on the side of the convexity. No mention was made of the intervertebral discs and posterior vertebral elements or the possibility of a scoliosis having been present. Gray (1973) also made reference to a lateral curvature and handedness by

suggesting that it is often present in the upper thoracic region with its convexity directed to the side of dominance. White (1971) reported that McCarver *et al.* (unpublished, 1970) found handedness to be of little consequence in that out of 22 left thoracic curves in observed IS patients, only 2 were left handed.

Other writers maintain that a natural lateral deviation of the upper thoracic spine exists and is due to the position of the thoracic aorta (Steindler, 1955). It appears significant that in the presence of vertebral osteophyte formation the left anterolateral aspects of the thoracic vertebral bodies are devoid of osteophyte occurrence (Culver, 1960; Nathan, 1962).

In scoliosis lateral curvatures can also be present in the lumbar and cervical regions of the vertebral column although their occurrence is less common (Moe *et al.* 1978). It is rather interesting in view of past information that Green (1981) also maintained that a lateral curve of the vertebral column is always pathological.

The vertebral column has often been designated as "the spine" or the "spinal column" (Hollinshead and Jenkins, 1981) while the associated posterior musculature has acquired such labels as the "true back muscles" (Hollinshead and Jenkins, 1981), "extensors or muscles of the back" (Hartman and Straus, 1933; Basmajian, 1982), "deep back muscles" (Gray, 1973; Woodburne, 1983), "intrinsic" (Anson, 1966; Gray, 1973) or "posterior mass of longitudinal

extensor muscles" (Last, 1978). In this thesis the nomenclature used will be "the vertebral column" and "deep and/or superficial paravertebral" musculature.

The vertebral column, likened to a multiaxial joint, is capable of movement about three degrees of freedom and the muscles most intimately associated with and partly controlling these movements are the paravertebral muscles. Regardless of the species this posterior mass of paravertebral musculature represents the deep or intrinsic muscles of the vertebral column, derived from the dorsal divisions of the embryonic myotomes and are innervated by the dorsal rami of adjacent spinal nerves. They consist of a complex group of muscles running in close apposition to and collectively controlling the whole length of the column. The paravertebral muscles are situated deep to the thoracolumbar fascia and more superficial muscle layers associated with the upper limb and include complex extensors and rotators of the head and neck as well as the rest of the column where such movement is allowed. The muscle layers are composed of multilayered individual units which crisscross each other in different planes and at various levels. Collectively, they control and move the spine due to their expansive attachments from pelvis to skull. With the exception of Last (1978) most authors subdivide this posterior mass of musculature into superficial and deep groups, naming them erector spinae and transversospinal muscles respectively. Last (1978) prefers to categorize both groups as "The

Erector Spinae Muscle". The most superficial muscle layers controlling the shoulder girdle are not considered in this study; only those intrinsic muscles, categorized below, and pertinent to movements specifically of the vertebrae in a gross and individual manner are included.

Although there are differing descriptions in the literature as to the most specific muscle attachments of human paravertebral muscle (Jackson, 1914, Gray, 1973; Warfel, 1973; Hollinshead, 1974; Last, 1978; Bogduk, 1980; Hollinshead and Jenkins, 1981; O'Rahilly, 1983; Woodburne, 1983) they are relatively minor and the general makeup of this muscle mass is fairly constant.

The paravertebral muscles are conveniently subdivided into SUPERFICIAL and DEEP groups. They consist of the following muscles:

SUPERFICIAL - Erector Spinae (Sacrospinalis)

1. Iliocostalis - lumborum, thoracis, cervicis
2. Longissimus - lumborum, thoracis, cervicis, capitis
3. Spinalis - thoracis, cervicis, capitis

DEEP - Transversospinalis

1. Semispinalis - thoracis, cervicis, capitis
2. Multifidus - entire length of spine
3. Rotatores - best developed in thoracic region

- Associated

1. Interspinalis -in pairs between spinous processes
2. Intertransversarii - lumbar, thoracic, cervical

For a detailed account of human muscle attachments see Appendix A.

C. MUSCLE FIBER CHARACTERISTICS, PROPORTION, AND SIZE

FIBER TYPES

As mammalian muscle develops, the various fiber types normally differentiate corresponding to the stage of maturity of that subject and according to the species involved. Differentiation is complete when a particular muscle in a particular species is composed of fibers with differing contractile and metabolic properties which will meet the specific functional requirements of that muscle (Close, 1972; Burke and Edgerton, 1975; Armstrong *et al.* 1982).

The histochemistry of developing muscles has been described by several workers in a number of different species including the cat, rat, guinea pig, hamster and rabbit (Dubowitz, 1963, 1965, 1968), mouse (Wirsen and Larsson, 1964), pig (Cooper *et al.* 1970; Ashmore *et al.* 1973), lamb (Ashmore, 1972b), monkey (Beatty *et al.* 1966, 1967) and human (Dubowitz, 1963, 1965, 1966, 1968; Fenichel, 1963, 1966; Goldspink, 1972; Colling-Saltin, 1978; Stickland, 1981). Histochemically limb muscle in humans is essentially like that of all other mammals (Burke and

Edgerton, 1975).

Developing muscle differentiates at different times prenatally and postnatally in human and animals. Dubowitz (1963, 1965, 1966) established a sequence of events for the process of differentiation in developing human muscle. He reported that in various muscles taken from 36 newborn, premature, and full term infants, fibers are fully differentiated into two basic fiber types, as in the adult. With the exception of 1 premature infant of 26 weeks gestation in his study the distribution of type I and II fibers was approximately equal. Other fetuses, obtained at abortion (both therapeutic and spontaneous) ranging in gestation from 20-26 weeks, demonstrated two fiber types but there was a disproportion between the two types with the majority belonging to the type II category. The proportion of type I fibers ranged from about 3-10% of the total and were in the upper range of fiber diameter. The remaining fetuses (12-18 weeks) did not show this pattern of muscle fiber difference.

Dubowitz (1965) determined the following sequence of muscle fiber differentiation:

PHASE 1: 12-18 weeks gestation: Muscle cells were without consistent enzyme content; no subdivision was apparent for type I and II fibers.

PHASE 2: 20-26 weeks gestation: Subdivision into type I and II was possible; type I represented only a small proportion of the total number of fibers and were larger in

diameter.

PHASE 3: 30 weeks on: Subdivision into Type I and II fibers was readily apparent demonstrating a "checkerboard" pattern; there was an equal distribution in proportion of the two fiber types.

Fenichel (1966) studied biceps brachii and thigh muscles in fetuses which were obtained from therapeutic abortions and of gestational ages varying from 5-20 weeks. He determined that fiber typing was not possible during the period from 5-8 weeks due to uniformly intense mitochondria and too few myofibrils. During the period 8-10 weeks, myotubes were the predominant cell with type II fibers (15-20 μ m diameter) more numerous than the smaller type I (5-10 μ m). Between 10 and 20 weeks myofibrillar formation was progressing and mature myofibers were evident. Fiber typing was possible in many muscle cells at this stage of development. Finally at 20 weeks the muscle had a mature appearance with fiber types being equal in number but the size relationship had reversed in that type I fibers were now bigger. From 20 weeks on, Fenichel (1966) agreed with the sequence of events presented by Dubowitz (1965) as to the development of the two histochemical fiber types present in human muscle, demonstrating that the two muscle fiber types develop as separate populations. According to Fenichel (1966) the Adenosine Triphosphatase (ATPase) reaction is the most useful histochemical technique for fiber typing during the early weeks of gestation.

Beatty *et al.* (1967) investigated by histologic, histochemical and analytical techniques, the stage of fetal development at which differentiation of muscle in the rhesus monkey became apparent. Their samples consisted of limb muscles (brachioradialis and soleus) taken from fetal, neonatal and infant monkeys, stained for Succinate Dehydrogenase (SDH) activity. They observed that rhesus monkey muscle showed a pattern of differentiation similar to that of human adult muscle at approximately the same time (73% of term as with human muscle 75% of term). In these animals, SDH activity was lower in fetal and infant muscle as compared to adult muscle and SDH enzyme activity was higher in red than in white muscle, as early as 90 days fetal age. Their histochemical data agreed with that of Dubowitz (1965) in that the pattern of differentiation 17 weeks to term, was similar to that for human 30 weeks to term. Calculated on a basis of gestation period for each period for each specimen, correlation appears feasible.

Skeletal muscle fibers can ordinarily be classified into categories which can be repeatedly recognized on the basis of a variety of histochemical reactions or profiles. It has long been recognized that mature (differentiated) muscles are not homogeneous in the makeup of their fiber types (Brooke and Kaiser, 1970; Dubowitz and Brooke, 1973). Most skeletal muscles are composed of different fiber types and varying proportions of each fiber type may be present in different muscles. The further division of fibers into

sub-types is accomplished by observing the staining behavior of fiber types using histochemical methods (See Figure 1-1) involving three major groups of enzymes:

1. oxidative - the dehydrogenases: NADH-TR (NADH - tetrazolium reductase), SDH (Succinic), LDH (Lactic), AGDH (alpha-glycerophosphate).

2. hydrolases - ATPase (Adenosine Triphosphatase) and various esterases.

3. those connected with glycogen synthesis and breakdown such as Phosphorylase.

An outline of the methods used in this study can be referred to in Appendix B.

A variety of fiber classification schemes have been employed (Dubowitz and Pearse, 1960, 1973; Engel, 1962; Stein and Padykula, 1962; Padykula and Gauthier, 1967; Brooke and Kaiser, 1970; Guth and Samaha, 1969, 1970; Barnard *et al.* 1971; Peter *et al.* 1972; Prince *et al.* 1976) based on the use of histochemical procedures as an investigative tool to localize enzyme systems and other chemical constituents at a cellular level. In the present study the nomenclature and classification of muscle fiber types as presented by Dubowitz and Brooke (1973) and Brooke and Kaiser (1974) is used. See Figure 1-1.

Historically, fiber typing and nomenclature has changed and appears to have been influenced by the advances in histochemistry as well as personal choice. Classical terminology of red and white muscle fibers was related to

Figure 1-1. Histochemical Reaction in Human Muscle Fiber Types.*

Muscle Fiber Type	I	2A	2B	2C
Routine ATPase	○	●	●	●
ATPase preincubated pH 4.6	●	○	●	●
ATPase preincubated pH 4.3	●	○	○	⊕
NADH -TR	●	●	⊕	⊕
SDH	●	⊕	⊕	⊕
glycerophosphate -menadione linked	○	⊕	⊕	⊕
PAS	⊕ ●	●	●	⊕
Phosphorylase	⊕ ○	●	●	●

○ = 0 ⊕ = 1+ ⊕ = 2+ ● = 3+

*Taken from: - Brooke, M. and Kaiser, K.: *Use and Abuse of Muscle Histochemistry*, 1974.

- Dubowitz, V. and Brooke, M.: *Muscle Biopsy: A Modern Approach*, 1973.

the myoglobin content and subsequent color of the muscle fibers (Romanul, 1964). However, recent histochemical evidence indicates that there are more fiber types present

in mammalian muscle than simply red and white. As many as 8 different fiber types have been described (Romanul, 1964).

Stein and Padykula (1962) defined 3 fiber types (A, B, and C) based on the cytochemical distribution of SDH activity in the rat gastrocnemius. Correlated with the classical terminology they determined that the white fiber was represented by type A fiber and the red fiber was actually represented by 2 fiber types, B and C. Later, based on mitochondrial content and using EM, Padykula and Gauthier (1967) proposed 3 fiber types to replace the A-B-C nomenclature: white, intermediate and red. Other classifications emerged such as fast-twitch-white, fast-twitch-red, and slow-twitch-intermediate (Barnard *et al.* 1971); FF, FR and FS (Burke *et al.* 1971); α , β and $\alpha\beta$ (Guth and Samaha, 1969, 1970); ST, FTa and FTb (Saltin *et al.* 1977) to confuse the issue further.

Dubowitz and Pearse (1960) suggested that fibers which were rich in NADH-TR and poor in phosphorylase be called type I and fibers which were rich in phosphorylase but poor in NADH-TR, be called type II. It has been shown since then, that type II fibers are also rich in AGDH (Pearse, 1961) and in myofibrillar ATPase (Engel, 1962). Based on the histochemical ATPase reaction following alkaline preincubation (Engel, 1962), light staining fibers were classified as type I fibers and dark staining fibers were classified as type II fibers. Type I and II fibers were supposed to have slow and fast contraction velocities

respectively (Saltin *et al.* 1977) the terminology of which changed to slow-twitch (ST) and fast-twitch (FT) respectively (Gollnick *et al.* 1972; Costill *et al.* 1976a). These categories were further elaborated as fast-twitch-red, fast-twitch-white and slow-twitch-intermediate (Barnard *et al.* 1971). Because the myofibrillar ATPase activity was found to be dependent not only on pH sensitivity (Padykula and Herman, 1955) but on temperature (Khan *et al.* 1974) and staining times as well, Brooke and Kaiser (1970) proposed a 4 fiber classification scheme for human skeletal muscle retaining Engel's (1962) original findings. Based essentially on the pH labilities of the myofibrillar ATPase and basically retaining the type I and II division, Brooke and Kaiser (1970) subdivided type II fibers into subclasses IIA, IIB and IIC. This simple system of fiber type classification is routinely used today with the exception of type IIC fiber, found to be rare in mature human muscle (Dubowitz and Brooke, 1973). Brooke and Kaiser (1974) considered type IIC fiber to be an undifferentiated fiber, more predominant in immature muscle and considered the subdivision of type I fiber (using PAS and phosphorylase) as impractical. Dubowitz and Brooke (1973) support the use of an extended ATPase reaction for definition of type II subtypes and in addition recommend the use of NADH-TR in preference to phosphorylase as a means for comparison and spotting of pathological structural changes. Brooke and Kaiser (1974) also demonstrated gradations among the type II

fiber subclasses using histochemical staining intensities for NADH-TR, SDH and AGDH for reflecting different metabolic potentials. See Figure 1-1. At present the most popular method for fiber type nomenclature in humans is based on the ATPase reaction.

A study by Pette *et al.* (1980) investigated the activity of SDH along a single micro-dissected rat psoas muscle fiber. Their findings did not support earlier reports (Pool *et al.* 1979b) that the enzyme levels varied along the course of a muscle fiber. In addition, their results confirmed previous findings which determined that ATPase was uniformly distributed along muscle fibers.

One important limitation in fiber type differentiation that appears to persist in comparison of animal to human is the occurrence of fibers which do not fit into a major fiber type classification using using a single histochemical stain. Such fibers have been labelled "intermediates" (Dubowitz and Brooke, 1973) and complete histochemical profiles are necessary to determine their placement. For non-human skeletal muscles, fibers are commonly characterized as belonging to one of three groups based on their staining reaction to an oxidative stain in addition to myofibrillar ATPase (Peter *et al.* 1972). By using an oxidative stain such as NADH-TR, fast-twitch type II fibers are further subdivided into high oxidative and low oxidative groups. Based on these two stains and other biochemical characteristics, Peter *et al.* (1972) identified 3 fiber

types in guinea pigs and rabbits: SO - slow-twitching, oxidative; FG - fast-twitching, high glycolytic; FOG - fast twitching, high oxidative, high glycolytic. Advantages associated with this type of nomenclature are that the metabolic characteristics of the three groups can be identified in addition to the contractile properties. When using this classification on human muscle, Prince *et al.* (1977) reported that human muscle is composed of fiber types similar to those of lower mammals but are not as distinct when differentiating FOG fibers from FG.

Brooke and Kaiser (1974) favoured the use and elaboration of the basic two fiber-type classification system using ATPase for many animal muscles as well as normal adult human muscle and reported that the classification of Peter *et al.* (1972) is compatible with the type I, IIA, IIB, IIC system. In addition, a normal component of some animal muscle is the type IIC fiber, seen also in fetal muscle, abnormal muscle and reinnervating animal muscle. When comparing man with other species such as the cat, guinea pig or rat, Saltin *et al.* (1977) suggested that the muscle fiber most different in character and function may be the IIA (FTa and FOG) fiber. Saltin *et al.* (1977) cautioned against applying conclusions to more than the species studied but speculated that the following may be true: ST=SO=I; FTb=FG=IIB. Both Dubowitz and Brooke (1973) and Brooke and Kaiser (1974) maintained that correlation between the ATPase and NADH-TR reactions may be possible in

a given animal. However, when another animal or another muscle in the same animal is studied, the histochemical correlations may be different (Nemeth *et al.* 1979). It is the variation of type I fibers in their intensity of reaction to NADH-TR which cause the difficulties in that type IIA fibers may react more intensely than type I fibers in animals. Human muscle fiber types are much less complicated to classify than animal muscle fibers (Brooke and Kaiser, 1970; Burke and Tsairis, 1974; Essen *et al.* 1975). Furthermore, fiber typing based only on the NADH-TR and other oxidative enzyme techniques are subject to variation due to the metabolic state of that muscle at that time (Saltin *et al.* 1977). In light of the available information, a two-fiber classification system using the ATPase stain technique for identification of muscle fiber types and the NADH-TR stain technique for identification of structural changes seems most appropriate.

The ease of obtaining human muscle samples by the use of the needle biopsy technique has ignited the interest of many investigators involved in the field of sports medicine as a possible predictor of athletic success. Considerable variation in muscle fiber characteristics has been found in individuals undertaking various training activities (Saltin *et al.* 1977). Researchers are encouraged to be specific as to their methods, sites and muscles sampled (Saltin *et al.* 1977) and Tsairis (1974) is not in favour of needle biopsies because large enough samples cannot be obtained for proper

evaluation.

The functional activity of individual muscle fibers and their morphology can be correlated to enzyme systems and other chemical constituents of the fiber and may be profoundly influenced by nerve supply (Dubowitz, 1968). Heterogeneity is related closely to nerve supply (Stein and Padykula, 1962). Particular properties acquired by the muscle fibers of an identical group, innervated by branches of a single motor neuron are a result of the pattern of activity exerted on the fibers by the motor neuron. These properties can be histochemically transformed using long-term stimulation of opposite nature and frequency (Eisenberg and Salmons, 1981). Histochemical profiles can be altered by cross-innervation (Buller *et al.* 1960; Dubowitz, 1967; Barnard *et al.* 1970; Peter *et al.* 1972; Amphlett, 1975; Mommaerts *et al.* 1977), endurance training (Costill *et al.* 1979; MacDougall *et al.* 1979), removal of synergists (Salleo, 1980), and by changes occurring during the course of development and normal maturation (Burke and Tsairis, 1974).

Cross-innervation studies in the cat (Buller *et al.* 1960; Mommaerts *et al.* 1977) showed that by reversing the innervation to slow and fast muscles, the contractile properties of these muscles were largely reversed. Dubowitz (1967) and Mommaerts *et al.* (1977) determined that in cross-innervation the fast fibers assumed almost complete rapid transformation whereas in the slow fibers, the results

were less consistent with the counterpart being reached more slowly. Brooke and Kaiser (1974) agreed that each fiber type has its own separate innervation, individual nerves supplying motor units that are made up of muscle fibers of the same type. In re-innervation, therefore, there is type-grouping.

Eberstein and Goodgold (1968) described two fiber types in skeletal muscle (multifidus) as having very different mechanical and electrical properties as a result of a difference in twitch contraction time, resting potentials, tetanic responses and conduction velocities. Investigations by Kugelberg and Edstrom (1968) and Burke *et al.* (1975) have also shown that motor units are homogeneous suggesting a neural influence in the maintenance of fiber types. Factors such as impulse frequency, total number of impulses and response of the muscle, as well as neurotransmitter substances have all been reviewed (Hoyle, 1983). Robbins *et al.* (1969) determined that a correlation of physiological function and anatomical properties did not show a clear-cut association of these properties although Burke and Tsairis (1974) recognized a correlation between physiological and histochemical profiles in cat soleus and gastrocnemius muscles. However, Burke and Tsairis (1974) cautioned investigators against making ready assumptions concerning other muscles and other species particularly using histochemical data only. Histochemical profiles when correlated with differences in twitch-contraction times and

resistance to fatigue indicate that all of the muscle fibers belonging to a single muscle unit have more or less identical physiological properties (Gollnick *et al.* 1980).

In summary, characterization of individual fibers to determine the heterogeneity of muscle fibers in skeletal muscle of vertebrates is demonstrated most readily by using a histochemical approach (Dubowitz and Brooke, 1973). Normal skeletal muscle fibers when subjected to these histochemical procedures, demonstrate particular reactions and morphological structure. From these histochemical reactions certain anatomical, physiological and biochemical interpretations can be made.

Mammalian skeletal muscle, during the course of development, becomes specialized to perform different physiological tasks and have considerable powers of regeneration (Sloper and Partridge, 1980). Muscle fibers which are required to perform sustained contraction over a prolonged period of time are classified type I, demonstrate slow-twitch contractile characteristics, derive their energy mainly through oxidative metabolism and are not easily fatigued. Muscle fibers responsible for the execution of intermittent rapid movements over short periods with fast-twitch properties are classified as type II fibers. Some of the type II muscle fibers are dependent on an anaerobic energy metabolism and are readily fatigued (IIB) while others utilize both oxidative and glycolytic pathways and are less fatigueable (IIA). The capillary network in

close relation to individual fibers is more profuse around type I fibers (Dubowitz and Brooke, 1973).

Fiber type predominance has been interpreted as an excess of one fiber type, a normal distribution of type I to type II fibers being represented by a ratio of 1:2. Further considerations would be if type I fibers occurred in excess of 55% or type II fibers occurred in excess of 80% of the biopsy. If type IIA and IIB fibers are individually considered, then over 55% predominance of one type is considered excessive. Type I predominance has been associated with myopathies whereas type II predominance has been associated with motor neuron diseases (Dubowitz and Brooke, 1973; Dubowitz, 1978). Human muscles may contain 10% to 50% type IIC fibers after birth but these are reduced to less than 2% within the first year of life (Colling-Saltin, 1978). Type IIC fibers have been labelled "unclassified" and "undifferentiated".

Type I grouping may be a result of a conversion of histochemical properties of muscle fibers due to collateral branching. In contrast, type II grouping could be a reflection of loss by degeneration of type I muscle fibers (Morris, 1969). Fiber type deficiency is when less than 10% of the fibers represent a given fiber type; i.e. type I, IIA, IIB (Dubowitz and Brooke, 1973). Other muscle pathologies demonstrate degeneration and regeneration seen best by using hematoxylin and eosin (H & E) and Trichrome stains, or necrosis where fibers are faintly colored or pale

staining and frequently filled with phagocytes. The subdivision into two fiber types will usually give enough required information as to type grouping, type atrophy and interpretation of human muscle pathology (Dubowitz and Brooke, 1973).

PROPORTION AND SIZE

Stickland (1981) reported that the number of muscle fibers in a given muscle increases before birth until a genetically determined number for that muscle and species is attained and that in the human the final complement is determined before birth. Stickland (1981) noted that the total number of muscle fibers in the human rapidly increased up to about 22.5cm CR (to 165 days). Thereafter the rate diminished whereupon hyperplasia appeared to be replaced by hypertrophy of muscle fibers, contributing to the total muscle cross-sectional area although at 35cm CR (270 days) there was still a 6% contribution to muscle area from hyperplasia. Throughout the period studied (7.0 to 35.0cm CR) Stickland (1981) also noticed a decrease in intercellular space and the number of nuclei per unit area.

Komi *et al.* (1977) examined the vastus lateralis (VL) muscle in 31 pairs of monozygous (MZ) and dizygous (DZ) twins. He concluded that a significant genetic component was present in determining skeletal muscle fiber composition in that the MZ twins of both sexes had essentially identical muscle fiber composition as compared with the DZ twins. Bell *et al.* (1980) found that skeletal muscle differed minimally

between 6 year olds and that normally found in adults with the exception of a possible slightly greater capacity for oxidative metabolism.

Relative muscle fiber proportions (type I and II) vary between individuals within a given muscle (Burke and Edgerton, 1975) and numerous studies related to athletic power and endurance achievement seem to concur (Costill *et al.* 1976a, 1979; Prince *et al.* 1976; Saltin *et al.* 1977; Houston, 1978). Saltin *et al.* (1977) reported that although these variations existed and were greater in males, mean values for fiber type proportion were similar between the two sexes. Komi and Karlsson (1978) found muscle fiber distribution to be different between males and females and suggested that hormonal influences were indisputable as contributing factors. Quantitative assessment, using oxidative stains and following extreme specialized activity will alter muscle fiber proportions. Dubowitz (1968) suggested that fiber type proportions may vary not only between subjects but in different parts of a given muscle and that samples may be taken from those muscles not necessarily engaged in a given activity. Burke and Edgerton (1975) maintained that in man, unlike most other mammals, fiber type distribution is relatively homogeneous throughout the depth of the muscle. Saltin *et al.* (1977) agreed with this latter description citing Edgerton *et al.* (1975) and Johnson *et al.* (1973).

Few histochemical studies have been done on typical paravertebral muscle in humans (Polgar *et al.* 1973; Johnson *et al.* 1973; Fidler *et al.* 1975; Jowett *et al.* 1975) and animal (Spencer and Zorab, 1976). In IS a significant difference in fiber type proportions has been reported in the human paravertebral muscle on opposite sides of the column (Fidler *et al.* 1974; Jowett *et al.* 1975; Spencer and Eccles, 1976; Fidler *et al.* 1976; Yarom and Robin, 1979; Green 1981; Ford *et al.* 1983; Sahgal *et al.* 1983).

In cross-section the size of muscle fibers was stated as being closely correlated with energy metabolism (Romanul, 1964) whereas Brooke and Kaiser (1974) introduced gender as a factor by determining that type II fibers are larger in the human adult male than the female. Prince *et al.* (1977) concurred with this latter finding. Saltin *et al.* (1977) are in agreement that differences in muscle fiber size do exist between the sexes and that males have fibers with larger cross-sectional areas. In both sexes the mean cross-sectional area of type IIB fiber is smaller than type IIA:

males - type IIA > type IIB > type I

females - type I > type IIA > type IIB

Buchthal *et al.* (1974) reported that no consistent differences existed in diameter of different fiber types in man and therefore size was not suitable as a basis for classification. Comparison of human fiber type diameters with those of other small mammals showed that nomenclature

and species differences were accountable for most inconsistencies.

Atrophy, or a reduction in size of all or some of the muscle fibers in a sample, may occur due to disuse, deprivation of innervation or pathology. Therefore, reduced size of muscle fiber may have a physiological as well as a pathological basis and may involve together or singularly type I and II fibers. Selective type I atrophy is uncommon occurring in dystonias, myotonia, rheumatoid arthritis and some childhood diseases. Selective type II atrophy is very common to muscle pathology occurring in any disease in which muscle strength is impaired secondary to some remote problem. Type II atrophy also occurs in normal muscle tissue although the reason for this is unknown (Dubowitz and Brooke, 1973).

Hypertrophy, or an increase in size of type I and/or II muscle fibers may occur not only with repetitive and sustained exercise (physiological hypertrophy) as mentioned but also as compensation for functional loss in smaller atrophic fibers. Type-specific hypertrophy is less common (Dubowitz and Brooke, 1973) although type II hypertrophy is commonly seen as a rapid effect following exercise.

Several investigations have been conducted in an attempt to determine the pattern of muscle fiber type transformation. Hoyle (1983) speculated that some kind of physiological stimulus is responsible for the conversion of fiber types although the nature of the stimulus is unknown.

Costill *et al.* (1979) noted a change in fiber area ratio (VL) with endurance training whereby type IIA fibers appeared to convert to type I and/or type IIB fibers although no statistical changes in proportion were present.

AGING

The aging process occurs in all organ systems to varying degrees, at various times and rates of speed. Aging changes "are often complicated or rendered insignificant by those pathological lesions brought on by diseases to which the aged are exposed, and by secondary functional alterations" (Rubinstein, 1960). In a study of extraocular muscles, Rubinstein (1960) suggested that the changes of aging are probably fairly characteristic although the hormonal, genetic and state of development and training of particular muscles are of indeterminable importance. In general, Rubinstein (1960) described the characteristics of muscle cell aging as exhibiting: atrophy; increased intracellular pigment; ringed fibers; peripheral sarcoplasmic masses and central condensations of the myofibrils; loss of myofibrils and granular disintegration of sarcoplasm; marked inequality in size of muscle fibers; increased subfascial fat; intertwining obliquely running myofibrils; and loss of definition and of cross-striation with eventual disintegration. These changes were not restricted to the elderly (Rubinstein, 1960) and can be found for example in the external ocular muscles of the young.

Larsson *et al.* (1978) and Larsson (1978) determined the morphological, histochemical, biomechanical and functional changes in VL muscle with age in 55 healthy sedentary white collar worker males, aged 22 to 65 years. They determined that it was difficult to distinguish between changes due to aging and those due to other degenerative lesions. With age, there was a statistically significant increase in the percentage of type I fibers, resulting in a 41% and 55% distribution in the 20 to 29 and 60 to 65 year old groups respectively. Both the IIA and IIB percentage distributions decreased significantly (33%) although the type IIC fiber subtype remained unchanged (2 to 4%) and the fiber type distribution within the type II population did not vary with age. In addition, the subjects displayed a selective linear decrease in the average cross-sectional area of type II fibers with age but not with type I fibers. No significant change in ATPase activity occurred although oxidative capacity was found to be significantly higher in the young groups. Larsson (1978) suggested that these changes were indicative of the change in type II fiber content.

D. IDIOPATHIC SCOLIOSIS AND RELATED PARAVERTEBRAL MUSCULATURE

Three basic types of spinal deformity which occur are scoliosis, kyphosis and lordosis any of which may occur singly or in combination with one another. The Scoliosis Research Society has directed its efforts toward

standardizing a language and classification system for these spinal deformities according to magnitude, location, direction and etiology. Scoliosis, of importance in this study, accompanies many diseases and abnormalities.

By definition scoliosis is a lateral deviation of the vertebral column which is usually accompanied by vertebral rotation and may appear at any age during skeletal growth in otherwise healthy children. Scoliosis is classified as non-structural or functional if the curve is flexible and allows side-bending toward the side of the convexity. If the curve is rigid and fails to correct on side-bending it is defined as structural and the development of structural changes and their time of appearance varies. If progression occurs, the apical spinous processes deviate toward the side of the concavity and distortion of the rib cage on the side of the convexity produces a characteristic "rib hump".

Over 70% of all forms of lateral deviation of the spine are of idiopathic type (Harrington, 1977; Keim, 1979) which can be classified into 3 relatively well-defined periods of development according to age of onset (Moe *et al.* 1978):

A. Infantile: 0-3 years

1. Resolving

2. Progressive

B. Juvenile: 3-10 years

C. Adolescent: 10 years to maturity

Of particular interest to this study is the adolescent form of IS. Despite extensive research, the cause of scoliosis

remains obscure and in the adolescent form is more prevalent in girls (75-80%: Harrington, 1977; 70%: Keim, 1979).

Idiopathic scoliosis in adolescence commonly demonstrates a major structural curve, convex to the right in the thoracic region with minimal kyphosis, flatness or lordosis. In addition, a measurable rib hump of varying magnitude and rotation of the vertebral bodies on the side of the (Moe *et al.* 1978) convexity may be present as well as a less structural compensatory minor curve in the lumbar region of the spinal column. The major thoracic curve (typically from T4-5 to T12, L1) may appear first or simultaneously with the lumbar curve which extends to L4 or L5 vertebra. The apices of any curve pattern are usually variable in position although vertebrae commonly involved are T7, T8, T9, L1 and L2. Other curve patterns may occur although some with less frequency in the adolescent than others.

The etiology and pathogenesis of this characteristic deformity remains unknown although intensive research has suggested that the disease is probably genetically transmitted and multifactorial. In the past there have been many investigations related to familial (Wynne-Davies, 1968) and genetic disorders (Neurofibromatosis: Rezaian, 1976; Congenital dislocation of the hip: Hooper, 1980; Camptodactyly syndrome: Baraister, 1982), associated skeletal deformities (Loynes, 1972; Wynne-Davies *et al.* 1982; Papaioannou *et al.* 1982), pregnancy (Blount and

Mellencamp, 1980), intrauterine moulding (Lloyd-Roberts and Pilcher, 1965; Owen, 1974), metabolic errors (Misol, 1971; Smith and Francis, 1974; Beard *et al.* 1981), and neuromuscular disease (Dubowitz and Brooke, 1973; Engel *et al.* 1975; Dubowitz and Brooke, 1978).

The paravertebral muscles have been implicated by several investigators as a major causative factor in the production and progression of IS (Fidler *et al.* 1974; Fidler and Jowett, 1976; Spencer and Eccles, 1976; Spencer and Zorab, 1976; Yarom and Robin, 1979, 1979b; Low *et al.* 1983). Spinal deformities, independent of their etiology are susceptible to rapid progression during the period of adolescent growth spurt. Roaf (1958) suggests that foremost in the progression of scoliosis are dynamic and active rather than passive forces. Zorab (1977) claimed that muscle dysfunction in scoliosis is not a result of the scoliosis but a "disorder of connective tissue, perhaps muscle rather than collagen". Ponseti *et al.* (1976) in an extensive review of the literature determined that IS is a disorder of the spine exclusively, without generalized connective tissue involvement. Yarom and Robin (1979) suggested that the condition is a separate disease entity with possible central nervous system involvement.

TYPICAL PARAVERTEBRAL MUSCLE

Although much research has been directed toward the muscles in scoliosis, few studies have dealt specifically with the histological and histochemical analysis of

equivalent superficial and deep muscles on both sides of the vertebral column at the same specific predetermined sites.

Only a few histochemical studies of "normal" human paravertebral muscles have been made (Sulemana and Suchenwirth, 1972; Johnson *et al.* 1973; Polgar *et al.* 1973; Fidgett *et al.* 1975) and these include only general information often accompanied by an imprecise definition of the site of sampling often confined to one side of the vertebral column. Mean values for size and proportion of muscle fiber types have been compiled by these researchers and are referred to in Tables 4-1 and 4-2.

Unfortunately there is no simple way in which paravertebral muscle samples at predetermined sites and depths can be obtained from healthy individuals and which can be used to produce normal standards. Most samples are obtained in various studies either from autopsy material or from patients undergoing spinal operation for lumbar disc disorders. Certainly, numerous histochemical studies have been conducted on other human muscles and a variety of mammalian muscles. Generalities may be extended to paravertebral muscle.

Brooke and Kaiser (1970) determined that little difference was present in average diameter of type I and type II fibers in any normal human muscle. Sulemana and Suchenwirth (1972) examined the "erector spinae" muscles in 11 autopsy subjects without previous known disease, ranging in age from 22 to 73 years (9 males, 2 females; mean age

58.0 years), biopsy samples being taken soon after death. No details as to site or depth of sample were noted. The type I fibers were not only larger than type II fibers (mean diameter=68.7:65.7) but were significantly more numerous than type II fibers (mean %=62:38) respectively. Variations existed in fiber type proportion between individuals (type I: 50-60% and type II: 30-50%). Sulemana and Suchenwirth (1972) suggested that these differences may be due to some unknown factors including post mortem changes although they did appear to be representative of the physiological functions performed.

Other investigators (Johnson *et al.* 1973; Polgar *et al.* 1973) examined both superficial and deep paravertebral muscle in 6 subjects on one side only at L4-5 vertebral region with similar results. In the superficial group, type I fibers represented 58.4% of the fiber type proportion with a mean fiber size of 60 μ m compared to type II fibers which had a mean fiber size of 57 μ m. The distribution of fiber type I was not as pronounced in the deep group (54.9%) although the mean fiber size of type I and II was significantly different (61.6 and 53.6 μ m). The figures for the superficial group, although slightly less, accord fairly well with Sulemana and Suchenwirth (1972).

Fidler *et al.* (1975) used samples of multifidus from 17 patients with lumbar spinal derangements and 3 autopsy specimens (mean age 34.8 years) as typical paravertebral muscle with typical mosaic pattern. They determined that

multifidus was composed of mainly type I fibers (66.6%) and that the size of type I and II fibers was 73.4 μ m and 59.4 μ m. No grouping of fiber types was evident in any case and although type I fibers appeared of normal outline, type II fibers were markedly angular in some cases. These investigators also analysed the two fiber types by dividing the subjects into 2 groups according to age: group 1 - 15 to 32 years; group 2 - 35 to 58 years. With age, there was a tendency for type I fibers to increase in proportion but not markedly in size whereas the type II fibers decreased in size. Fiber size displayed increased variation and in contrast to Jennekens *et al.* (1971, 1972) markedly atrophic fibers, target fibers, core fibers and other intracellular irregularities were not found. Fidler *et al.* (1975) suggested that these results indicate that the multifidi assume an increasingly postural role with age and with disabling lesions of the lumbar spine.

Spencer and Eccles (1976) conducted a similar study on 4 patients and compared measuring techniques of type I and II fiber sizes manually and with a computer-controlled microscope. The comparison of sizes measured by these two methods correlated well with one another. For type I fibers the diameter as measured by machine was 49 μ m and by hand 48.2 μ m whereas for type II fibers the figures were 47.03 μ m and 47.4 μ m. No signs of muscle disorder were apparent in their subjects.

Green (1981) obtained muscle biopsies at operation for laminectomy for lumbar disc lesions, from 4 male patients, aged 22 to 30 years. Specimens were taken from 1 region on each side of the spine but no details are available as to specific site or depth. Although the size of the fiber types was not determined, the distribution of type I fiber was 49.3% (37.4 to 62.2%) representing an almost equal heterogeneous population but a lower percentage than for other investigations mentioned.

Using muscle samples from patients with lumbar spinal derangement as typical paravertebral muscle could be questioned (Fidler *et al.* 1975) in that fiber type, size and strength of muscle may be altered in the presence of abnormal neurological signs so typical of disc prolapse. As well, obstruction of surrounding vascular and neurological functions may result in deviations from the normal. It could be supposed that musculature situated on the side of the lesion is unhealthy, being affected by the offending disc. These considerations will be discussed in subsequent sections of this thesis.

SCOLIOTIC PARAVERTEBRAL MUSCLE

Studies dealing specifically with the histological and histochemical analysis of muscle in IS suggest the presence of abnormalities in muscle fibers and asymmetry in fiber type proportions at the site of the major curvature. Fidler *et al.* (1974) consistently demonstrated more type I muscle fibers on the convex side of the curvature at the apex in

multifidus although a normal mosaic distribution was retained. This preponderance of type I fibers was interpreted as being a sign of increased strength resulting in deformation of the spinal column. No signs of denervation or myopathy were detected and no significant difference existed in fiber size between the two sides of the curve. These findings were supported by Spencer (1974) and Spencer and Eccles (1976) both groups interpreting this predominance in distribution and not in size of type I fibers as an indication of sustained (tonic) muscle action. In addition to fiber type predominance in IS, Fidler and Jowett (1976) found the multifidus muscle to be shorter on the convex side of the curve, particularly at the apex.

Hoppenfeld (1974) examined 25 cases of IS (8 to 17 years; 21 females and 4 males) in a preliminary study using histochemistry and EMG. Although many difficulties appear to have been encountered in obtaining controls, they did observe that the scoliotics had a marked increase in type I fibers and no abnormal EMG findings except for an increase in number of motor units on the convex side in accordance with Zuk (1962) and Riddle and Roaf (1955). Hoppenfeld (1974) felt that "idiopathic" scoliosis could merely be a manifestation of a more widespread neuropathic process.

Spencer and Zorab (1976) sampled 3 sites of the erector spinae muscles (above, below and at the apex) on both sides of the major curvature and found, in addition to type I fiber predominance, many abnormalities and pathological

changes indicative of denervation and neuropathy although not on one side or at one site more than another. These consisted of large variations in fiber size, fiber type grouping, whorling or "moth-eaten" fibers, isolated giant or coil fibers, target fibers, central nuclei and central unstained core fibers. An accompanying study (Spencer and Zorab, 1976) which compared muscle taken from normal and scoliosis-induced rabbits by plaster cast technique showed no difference in fiber type proportions between the 2 groups, suggesting that asymmetry of fiber type proportions was not a consequence of the scoliosis but a contributory factor.

Yarom and Robin (1979) noticed several abnormalities in muscle fibers as well and observed an imbalance in fiber types with the presence of type I atrophy on the concave side of the curve in the deep paravertebral muscles and a general decrease in the proportion of type II. In addition, Yarom and Robin (1979, 1979b) and Yarom *et al.* (1982) examined peripherally situated muscles and found that 2 usually mixed muscles, the gluteus maximus and the concave deltoid consistently demonstrated "hypotrophy" of both fiber types and an atrophy of type I fibers on the concave side, although of milder form than compared to the paravertebral muscles. Although other muscles were examined (quadriceps, iliopsoas, trapezius) they were generally variable in composition or with possibly a slight increase in type I fibers. Yarom and Robin (1979, 1979b) and Yarom *et al.*

(1982) interpreted these findings as being indicative of a generalized neuromuscular disorder unrelated to age, sex or degree of curvature, the possible primary defect being situated in the muscles and/or the motor unit or the brain. They also proposed that an injury during fetal life may be exacerbated postnatally by growth and sex hormone influences leading to propagation of the deformity.

Green (1981) examined paravertebral muscle biopsy samples taken from 7 patients with IS (6 females, 1 male; 12 to 14 years; 5 right, 2 left) at 3 sites on both sides of the column: level of the 1st neutral vertebra proximal to the curve; apex of the curve and level of the 1st neutral vertebra distal to the curve. No other sampling details were reported. Using haematoxylin and eosin (H & E) stained sections, no evidence of neuropathy or myopathy was present although sections stained with Sudan Black B and PAS showed lipid to be distributed throughout the fibers, and more glycogen to be present in type I fibers. In accordance with other investigators ATPase demonstrated a generally high proportion of type I fibers and a 9.1% higher proportion of type I fibers on the convex side at the apex of the curve. At the other sites, no significant differences were observed in fiber type proportion between convex and concave sides. Green (1981) was unable to determine fiber type proportions using NADH.

The histochemical and morphologic changes occurring in the paravertebral muscle and gluteus maximus muscle of

patients with IS (11 females, 4 males; 8 to 17 years) with curves greater than 40° were also examined by Sahgal *et al.* (1983). Samples were taken at the apex of the curve although pertinent details are lacking. These investigators found multiple abnormalities on both sides of the curve including variation in fiber size, central nuclei and splitting of fibers. The gluteus maximus showed similar abnormalities and a high proportion of type I fibers although it was not specified whether one or both sides were sampled. The results presented by Sahgal *et al.* (1983), using ATPase, are variable and confusing. On the concave side, a very low percentage of type II fibers was found in 9 subjects, 4 cases did not show significant differences between the 2 fiber types and 1 case showed a significantly greater proportion of type II fibers. On the convex side, 8 cases demonstrated a low percentage of type II fibers, significantly so in 5 cases (although not significant between sides) and the one case with a high proportion of type II fibers on the concave side, also demonstrated a high percentage of type II fibers on the convex side. The mean fiber diameter of type I/II fibers was slightly smaller on the concave side but they were not significantly different from those of the convex side. In general, Sahgal *et al.* (1983) found that both sides at the apex of the curve were affected to different extents in their subjects and suggested a primary muscle disorder. These inconclusive results may be due to sampling differences, age, or variable

technique. Furthermore, not all subjects were accounted for in their study.

ANIMAL MODEL

Several attempts have been made to induce scoliosis in experimental animals mainly for the purpose of arriving at some reasonable explanation as to the mechanism responsible for the deformity in man.

Most methods have been operative with the general principle being to alter longitudinal growth of the column unilaterally (Hakkarainen, 1981) or cause muscle imbalance in paravertebral muscles (Bagnall *et al.* 1983). Sevastikoglou (1978) and Hakkarainen (1981) have reviewed the various methods which have induced slight scoliosis and which include: muscle transection, excision or cauterization; muscle and ligamentous transection or excision; muscle denervation; rib resection; spinal fixation; electrical stimulation; vertebral growth retardation; operations on vertebrae; radiation; other methods such as labyrinthine stimulation or ablation, pleural damage, and disarticulation or provoked luxation of an extremity.

Although all vertebrates possess more or less the same spinal architecture, only man loads his spinal column vertically. This fact presents many difficulties when attempting to create an animal model for experimental purposes. Modification of the current treatment and/or assessment of new treatments for IS will require preliminary

verification on an animal model. Beatty *et al.* (1967) determined by histologic, histochemical and analytical techniques that the rhesus monkey compared well with the human in fetal development and Van Wagenen and Catchpole (1965) similarly determined that it was suitable for growth and development. Therefore, it seems logical that the rhesus monkey be given consideration as a model for further studies in IS if its morphological and histochemical makeup is satisfactory.

STATEMENT OF OBJECTIVES

1. To produce a histological and histochemical profile for the paravertebral musculature associated with human lumbar disc dysfunction, idiopathic scoliosis, and the rhesus monkey.

2. To specifically sample equivalent superficial and deep paravertebral musculature on both sides of the vertebral column in all subjects.

3. To determine if the paravertebral musculature in subjects with straight spines, although suffering disc dysfunction, can be considered as typical.

4. To determine the effect of age and gender on muscle fiber characteristics in human subjects with straight spines.

5. To determine differences, if any, in paravertebral muscle fiber characteristics associated with idiopathic scoliosis.

6. To compare and relate muscle fiber characteristics found in the paravertebral musculature of rhesus monkeys with those of the human to determine its potential as an animal model for the study of scoliosis.

II. METHODS AND MATERIALS

Human muscle samples were collected from the Royal Alexandra Hospital, Edmonton, over a period of about 1 year and according to the availability of suitable material. The entire procedure had been reviewed and approved by the Hospital Ethical Review Committee following which it was necessary for the patients to read and sign a consent form allowing biopsies to be taken. A copy of this form is included at the end of this section. Hospital records for the patient were consulted and personal information as well as that pertaining to the history and physical findings perceived by the physician were documented.

Running concurrently was the sampling of monkey paravertebral muscle which again depended upon the availability of specimens. Other investigators were using the monkey for studies related to cerebral vasospasm and the only medications delivered were intravenous sodium pentobarbital and procaine penicillin, according to body weight (Espinosa *et al.* 1982).

A. LUMBAR DISC DISORDER

Four muscle biopsy samples were taken at operation for spinal fusion from each of nineteen patients suffering from lumbar spinal derangement. The subjects ranged in age from 28 to 73 years (mean age 45.3 years), included 12 males and 7 females and had a relatively brief history of spinal

dysfunction, all except one being for less than one year (some as short as three weeks). On clinical examination all of the patients exhibited some positive signs of nerve root involvement such as pain and disability. In addition to these signs and symptoms 8 had diminished reflexes, 4 had muscle weakness with anesthesia and 3 demonstrated anesthesia alone occurring singularly or together.

In each case the investigations were done in parallel on corresponding muscles from both sides of the vertebral column. More specifically, muscle samples were consistently taken in each case from identical areas of equivalent superficial (Sacrospinalis) and deep (Multifidus) muscles.

After reflection of the dorsolumbar fascia on both sides of the vertebral column the biopsies were taken by the surgeon from the following sites:

sacrospinalis - 1cm. lateral to the tip of the
spinous process of L5 just deep
to the dorsolumbar fascia.

multifidus - 1cm. from the inferior border of
the lamina of L5.

B. ADOLESCENT IDIOPATHIC SCOLIOSIS

Twelve muscle biopsy samples were taken at operation for spinal instrumentation from each of seven patients suffering from idiopathic scoliosis and ranging in age from 11 to 22 years (mean age 14.3 years). Of the subjects one

was male and all but one exhibited right thoracic curves.

Care was taken during surgery to ensure that muscle samples were consistently taken in each case from identical areas of equivalent superficial and deep muscle groups on both sides of the major vertebral curvature.

After reflection of the most superficial limb girdle musculature in the posterior thoracic region the biopsies were taken by the surgeon from the following sites:

longissimus thoracis - immediately adjacent and lateral to the vertebrae at the level of the apex of the primary curve (T8 to T11 levels) and similarly two vertebral levels above and below the apex.

multifidus/rotatores - 1cm. from the inferior border of the lamina at the apex (T8 to T11 levels) of the primary curve and similarly two vertebral levels above and below the apex.

C. RHESUS MONKEY

Twelve muscle biopsy samples were taken from each of five adult female rhesus monkeys within 2 hours of sacrifice. In each case the samples were consistently taken from identical areas of equivalent superficial and deep

spinal musculature on both sides of the vertebral column.

After reflection of the most superficial pectoral girdle musculature and lumbodorsal fascia, the biopsies were taken by the author from the following sites:

longissimus - immediately adjacent to the
vertebrae at the level of
thoracic 3, 8 and lumbar 3.

multifidus/rotatores - immediately adjacent to the
laminae of the vertebrae at
the level of thoracic 3, 8
and lumbar 3.

All human and monkey muscle samples were oriented so that cross-sections could be taken, were immediately supported in agar blocks mounted on cork with OCT, and immersed in isopentane (-160° C) precooled in liquid nitrogen. Sections were cut in a transverse plane at $10\mu\text{m}$ with a cryostat microtome maintained at -20° C and subsequently subjected to the following histological and histochemical staining procedures:

1. Trichrome (Masson's, 1968)

The Trichrome stain technique was used for the selective demonstration of muscle fibers, collagen and nuclei. Application of this procedure stains the muscle fibers a pinkish-red, the collagen a light green, and the nuclei blue-black. See Plates H-1A, H-2A and H-3A. The morphology of the muscle fibers can be clearly demonstrated

and any abnormal structures such as internal nuclei, if present.

2. NADH (Dubowitz and Brooke, 1973)

A histochemical procedure used to demonstrate, if present, various structural changes in muscle such as "moth-eaten" or enzyme-deficient cores in central core disease which are not usually apparent with routine histological stains. Varying degrees of blue color are observed at the site of the enzyme activity. See Plates H-1B, H-2B and H-3B.

3. Adenosine Triphosphatase (Guth and Samaha, 1969)

A histochemical procedure whereby a series of reactions result in the production of an end product being deposited at the site of the ATPase enzyme activity, giving a mosaic or checker-board appearance to the muscle section. Used to demonstrate specific fiber types in a section, based on the enzyme reaction, or selective fiber type involvement in particular disease processes. As ATPase is pH dependent great care must be taken with the accuracy of the pH used in preincubation solutions. Visual rating by light microscopy of the intensity and pattern of reaction for ATPase permits easy categorization of the fibers (according to the classification of Dubowitz and Pearse, 1960; Dubowitz and Brooke, 1973) into types I (low intensity) and II (high intensity). See Plates H-1C, H-2C and H-3C. The ATPase reaction is considered more stable than other metabolic enzymes for the differentiation of fiber types and therefore

is considered the method of choice for muscle fiber typing. An account of the specific stain techniques and procedures employed can be found in Appendix B.

Assessment of the relative proportions of fiber types was made from photomicrographs taken of randomly selected areas on transverse sections and avoiding the periphery of the biopsy. The size of each fiber was determined by measuring the "lesser fiber diameter" (Dubowitz and Brooke, 1973) using concentric rings inscribed on a clear thin plastic sheet. All measurements were undertaken by one observer to alleviate variable interobserver interpretation.

The mean number of fibers analysed in muscle biopsies taken from human lumbar disc disorder patients was 65, and from idiopathic scoliosis patients was 78. The mean number of fibers analysed in muscle biopsies taken from rhesus monkey paravertebral musculature was 84. A previous project (Toman, 1979) had determined that this was sufficient to produce a mean value within the 5% confidence limits of the final mean for the specimen.

Yarom and Robin (1979) attempted to combine the muscle cell characteristics of type and size by introducing a strength factor. They multiplied the percentages of type I and II fibers by the square of the average diameter of each fiber. In this study it was preferred to calculate the actual mean area of the transected muscle cell rather than merely squaring the average diameter. A strength factor component for each muscle specimen and fiber type was

calculated using the formula:

$$\text{strength factor} = \% \text{fiber type} \times \text{mean fiber area}$$

The establishment of a strength factor component for each muscle introduced a method of combining the measurements into a functional concept. Therefore, the mean fiber size, percentage of fiber type and strength factor were used to define the muscle fiber characteristics.

D. DATA ANALYSIS

The data were analysed by comparing measurements taken of the superficial and deep muscle from both sides of the vertebral column in the following manner:

Lumbar disc disorder - affected side (side of the protrusion) with the unaffected side (n=18; 9 left and 9 right)
 - one side with the other side (left with right) (n=19; 9 left, 9 right and 1 central)

Further analysis was made by separating the data into groups based on sex and age; there were 5 young males (less than 40 years; mean age 30.4 years), 7 older males (greater than 40 years; mean age 48.6 years), and 7 older females (greater than 40 years; mean age 52.7 years). There were no young female subjects in our sample.

Idiopathic scoliosis - side of the concavity
with the side of the
convexity (n=7)

Rhesus monkey - one side with the other
side (left with right; n=5)

Comparisons were made using paired students t-tests with p less than or equal to 0.05. This was accomplished by feeding in raw data to The University of Alberta Michigan Terminal System which possesses a computer program for processing statistical data. The Statistical Package for Social Survey data (SPSS) was created out of a set of routines to analyze social survey data and has its own common language. It produces frequency listings, cross tabulations, correlations, and regression analysis when an entire "batch" of commands are submitted, to be processed by an Amdahl computer system. Once a batch of commands is submitted, no user intervention is allowed and the requested information is processed accordingly and executed by paper documentation.

For reasons of space conservation, tables compiling the statistical raw data collected are presented in the following Appendices:

Appendix C - Lumbar Disc Disorder: Affected vs Nonaffected

Sides of the Vertebral Column

Appendix D - Lumbar Disc Disorder: Left vs Right Sides

of the Vertebral Column

Appendix E - Adolescent Idiopathic Scoliosis: Concave vs
Convex Sides of the Vertebral Column

Appendix G - Rhesus Monkey: Left vs Right Sides
of the Vertebral Column

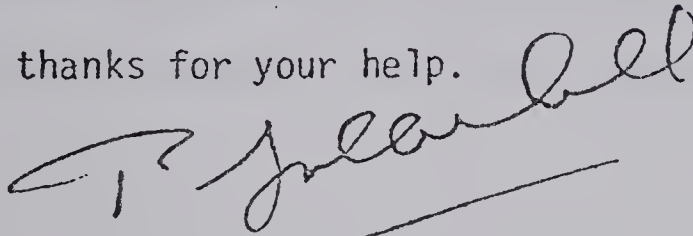
Dear

We would like your permission to perform a small additional procedure during your back surgery. This does not involve any extra incision or dissection.

As you know, during back surgery, in order to approach the vertebrae we separate the muscles temporarily from the bone. When we do this the edge of the large back muscles is seen and we wish to remove two small pieces of muscle measuring approximately 1/2"x1/8" from each side. We are sure that this will not cause you any additional pain or disability.

The removed muscle will be studied microscopically. This will enable us to give an opinion regarding the status of your back muscles. As we have no reason to think that you have any muscle problem the main purpose of the biopsy is to increase our knowledge of back muscle and to further our research into the cause of spinal curvatures of children.

Many thanks for your help.



B. J. GREENHILL, F.R.C.S.(C).

I give permission for Doctor Greenhill to perform muscle biopsy during my back operation, as explained above.

SIGNED

WITNESSED

DATED

III. RESULTS AND OBSERVATIONS

In all sections, no difficulty was encountered in distinguishing those fibers exhibiting a light and dark staining reaction for ATPase (pH 10.4 and 4.3) as cell boundaries and color contrast were clearly visible. As indicated in the Materials and Methods, care was taken to ensure proper alignment of muscle fibers. When the cryostat sections were examined microscopically the vast majority of muscle fibers in each section were cut transversely. No sections were discarded because of muscle fiber obliquity or for any other reason.

In the following section the observations and findings will be presented according to the subject sampled, outlining the histological and histochemical results in each case.

A. LUMBAR DISC DISORDER

Analysis of the biopsy material revealed a clear mosaic pattern of distribution of muscle fibers and fiber types, as described by Dubowitz and Brooke (1973). In all but 2 subjects, a few central core fibers (Greenfield *et al.* 1957; Bethlem, 1970; Dubowitz and Brooke, 1973) were observed (approximately 1%) in sections stained with NADH. Grouping of one fiber type was found in 3 patients but only involved approximately 30% of the fibers and were not atrophic in appearance. A few very small type II muscle fibers,

suggestive of inactivity (Dubowitz and Brooke, 1973; Fidler *et al.* 1975) were seen in only 4 patients. It is important to note, however, that no particular pattern was apparent in the above findings in relation to either the site of sample or site and duration of the protrusion in the patients with lumbar disc disorder.

Affected versus Unaffected Sides

The data were arranged in groups representing the affected (side of the protrusion) and non-affected sides categorizing separately the superficial and deep muscle layers. Of the patients, 9 had disc protrusions to the right and 9 to the left. (The 1 patient with a diagnosed central protrusion was not included for comparison in this category.) Table 3-1 presents this comparison of muscle characteristics by offering the means and standard deviations of affected and non-affected sides for type I and II muscle fibers. As can be seen in Table 3-1, the mean proportions and sizes of type I and II fibers are very similar in equivalent superficial and deep groups relative to affected and non-affected sides of the vertebral column. None of these values was found to be significantly different showing that these muscle characteristics were not dependent on the side of the disc protrusion. Mean strength factor values were established for both type I and II fibers (Table 3-1). Again mean equivalent values were found to be

	AFFECTED		NONAFFECTED	
	SUPERFICIAL	DEEP	SUPERFICIAL	DEEP
	mean(sd)	mean(sd)	mean(sd)	mean(sd)
Percentage	59(20.5)	53(15.0)	53(14.1)	49(19.5)
Type I fibers				
Percentage	41(20.5)	47(15.0)	47(14.1)	51(19.5)
Type II fibers				
Size Type I fibers	56.6(11.7)	58.9(16.7)	62.0(12.9)	58.8(8.3)
Size Type II fibers	37.9(11.3)	42.5(14.2)	37.2(8.4)	40.8(12.8)
S.Factor TypeI fibers*	148(68)	142(79)	157(55)	144(62)
S.Factor TypeII fibers*	51(40)	80(71)	55(30)	80(69)

Table 3-1. Muscle fiber characteristics related
to side of disc protrusion. (N=18)

* = strength factor $\times 1/1000$

Size = μm

similar and proved to be not significantly different. This showed that the "weaker" side was not related to the side of the disc protrusion.

In Table 3-1 the actual individual differences in strength factor, and proportions and sizes of fiber types between affected and non-affected sides of the vertebral column are concealed. Large differences between the two sides of the vertebral column (unrelated to affected and non-affected sides) were often found. Table 3-2 in particular shows the mean difference in strength factor between the two sides of the vertebral column.

	SUPERFICIAL		DEEP	
	mean	sd	mean	sd
Type I fibers (x1/1000)	-9	73	-2	102
Type II fibers (x1/1000)	-4	39	-0.1	85

Table 3-2. Mean strength factor differences between affected and nonaffected sides of the vertebral column. (N=18).

It can clearly be seen from the standard deviations that large differences in strength factor of the musculature

can exist between the two sides of the vertebral column. Similar large differences were found for the proportions and sizes of fiber types. These differences appear to be unrelated to the disc problem as the statistical analysis proved that the differences were not significant.

Left versus Right Sides

The data were arranged in groups representing the left and right sides of the vertebral column, categorizing separately the superficial and deep muscle layers. Table 3-3 presents the mean values and standard deviations of the muscle characteristics for both sides of the vertebral column for all subjects.

Significant differences were found between the two sides of the column only in the percentage of fiber types in the superficial muscles, with a greater percentage of type I fibers being found on the left and the converse (a greater percentage of type II fibers) being found on the right. No other significant differences were found between equivalent measurements on the two sides of the column. It is of particular interest that type I fibers were always significantly larger than type II fibers.

The figures in Table 3-3 conceal the true differences in muscle fiber characteristics that existed when comparing left and right sides of the vertebral column within individual subjects. Often these differences were large.

	LEFT	RIGHT
	mean (sd)	mean (sd)
SUPERFICIAL		
Percentage Type I	+61 (18.8)	+50 (13.5)
Percentage Type II	*39 (18.8)	*50 (13.5)
Diameter Type I	#55.1 (14.3)	\$62.2 (9.3)
Diameter Type II	#36.3 (10.7)	\$37.9 (8.9)
Strength factor	140.8 (54.1)	158.8 (67.0)
Type I (x1/1000)		
Strength factor	44.7 (40.8)	59.4 (25.8)
Type II (x1/1000)		
DEEP		
Percentage Type I	47 (19.5)	55 (14.0)
Percentage Type II	53 (19.5)	45 (14.0)
Diameter Type I	x57.0 (7.8)	&57.6 (15.0)
Diameter Type II	x41.2 (11.1)	&43.7 (15.6)
Strength factor	129.3 (57.1)	150.6 (81.7)
Type I (x1/1000)		
Strength factor	82.2 (63.2)	83.0 (75.1)
Type II (x1/1000)		

Table 3-3. Mean values of muscle fiber characteristics
for left and right sides of the vertebral
column at L5. (N=19; Diameter= μ m)
(Significant differences are indicated
by similar symbols.)

	MEAN	S.D.
DIFFERENCE		
SUPERFICIAL		
Percentage Type I	11.4	23.4
Percentage Type II	-11.4	23.4
Diameter Type I	-7.2	15.0
Diameter Type II	-1.7	7.2
Strength factor	-18.0	68.8
Type I (x1/1000)		
Strength factor	-14.7	80.3
Type II (x1/1000)		
DEEP		
Percentage Type I	-7.6	21.8
Percentage Type II	7.6	21.8
Diameter Type I	-0.7	15.3
Diameter Type II	-2.5	17.0
Strength factor	-21.3	97.8
Type I (x1/1000)		
Strength factor	-0.7	83.9
Type II (x1/1000)		

Table 3-4. The mean differences of muscle characteristics between left and right sides of the vertebral column.
(N=19; Diameter= μ m)

Table 3-4 shows the mean differences of muscle characteristics between opposite sides of the vertebral column and the variation in the form of standard deviations. From the large standard deviations, it is clear that similar values for muscle fiber characteristics on both sides of the column are not a common finding.

In comparing the influence of age on paravertebral muscle fiber characteristics, the 12 male subjects were separated into two groups; one group consisted of five males younger than 40 years (mean age = 30.4) the other of 7 males older than 40 years (mean age = 48.6). The statistical analysis did not reveal any significant differences for any measurements taken between these two groups. This suggests that age does not influence the fiber characteristics of the paravertebral muscles studied.

When considering the influence of gender on paravertebral muscle fiber characteristics the 12 males (mean age = 41.1 years) were compared with the 7 females (mean age = 52.7 years). The results of this analysis are shown in Table 3-5. It is important to note that the size of type II fibers on the left side only, both superficial and deep, is significantly greater in males than in females. The reason for this significance seems to be the extremely small type II fibers found on the left side in the female group. Although the same fibers on the right side also appear to be small relative to the male group, the difference between the two groups is not sufficiently large to be significant.

	LEFT		RIGHT	
	Males	Females	Males	Females
SUPERFICIAL				
Percentage Type I	60(22.2)	65(11.6)	48(15.8)	54(8.2)
Percentage Type II	40(22.2)	35(11.6)	52(15.8)	46(8.2)
Size Type I	58(17.0)	50(6.4)	63(10.5)	61(7.3)
Size Type II	*41(9.1)	*28(8.1)	41(6.6)	33(10.5)
Strength factor	148(63.9)	128(31.3)	160(78.3)	157(47.4)
Type I (x1/1000)				
Strength factor	58(46.4)	22(11.4)	68(23.5)	45(24.5)
Type II (x1/1000)				
DEEP				
Percentage Type I	41(21.5)	58(9.3)	53(16.9)	58(6.7)
Percentage Type II	59(21.5)	42(9.3)	47(16.9)	42(6.7)
Size Type I	57(8.7)	57(6.8)	59(16.5)	60(18.2)
Size Type II	\$48(7.2)	\$30(7.4)	48(12.6)	37(18.9)
Strength factor	114(51.9)	154(60.0)	145(72.5)	169(97.1)
Type I (x1/1000)				
Strength factor	#112(62.0)	#32(13.3)	99(79.5)	56(63.7)
Type II (x1/1000)				

Table 3-5. Mean values (S.D. in brackets) of muscle characteristics for both sides of the vertebral column at L5 in Males and Females. (N=19; Size= μ m) (Significant differences are indicated by similar symbols.)

These relationships compare well with those of Brooke and Kaiser (1970) who found that type I fibers are roughly equal in size in the two sexes but type II fibers are much smaller in women. In the deep group this small size of type II fiber in the females, coupled with the low percentage, has produced a significant difference in strength factor between the male and female groups.

Again, the main cause appears to be the relatively small size of type II fibers. It is important to note here that of the 7 females, 3 had disc protrusions to the right and 4 to the left. Analysis of affected versus unaffected sides has suggested that the side of disc protrusion does not have an effect on the paravertebral muscle fiber characteristics (Tables 3-1 and 3-2) but even so this variable is accounted for with approximately equal numbers relative to the side of the protrusion.

B. ADOLESCENT IDIOPATHIC SCOLIOSIS

The data collected from the muscle biopsy samples are presented in Table 3-6. Mean values for muscle fiber characteristics on both concave and convex sides of the major scoliosis curve were grouped according to the site and level of the biopsy.

Histological examination revealed generally well formed muscle fibers in all samples with normal well-defined perimeters, nuclei and intercellular material. Occasionally minimal atrophy of type II fibers was apparent; however

	ABOVE		APEX		BELOW	
	CONCAVE	CONVEX	CONCAVE	CONVEX	CONCAVE	CONVEX
	mean (sd)	mean (sd)	mean (sd)	mean (sd)	mean (sd)	mean (sd)
SUPERFICIAL						
% Type I	57 * (10.1)	74 (8.8)	61 (16.4)	67 (6.1)	57 * (15.1)	67 (15.5)
% Type II	43 * (6.8)	26 (12.1)	39 (10.0)	33 (5.6)	43 * (8.1)	33 (8.2)
Size Type I	43.4 (7.6)	48.9 (9.9)	45.7 (7.5)	50.8 (3.9)	41.2 * (6.0)	54.8 (9.5)
Size Type II	40.6 (6.8)	41.1 (12.1)	38.9 (10.0)	41.0 (5.6)	35.6 (8.1)	32.8 (8.2)
S.Factor I (x1/1000)	88.2 * (36.6)	143.5 (53.3)	96.7 * (27.7)	135.6 (23.4)	80.8 * (41.1)	164.1 (74.0)
S.Factor II (x1/1000)	56.8 (27.9)	35.8 (24.9)	48.3 (29.6)	45.8 (17.1)	40.8 (18.6)	29.7 (21.2)
DEEP						
% Type I	59 (13.9)	64 (10.0)	56 * (16.6)	70 (11.4)	55 (12.6)	60 (12.4)
% Type II	41 (9.8)	36 (6.1)	44 * (11.3)	30 (10.1)	45 (8.5)	40 (10.5)
Size Type I	46.4 (8.6)	49.2 (5.4)	44.9 * (5.9)	52.8 (9.2)	50.3 (11.6)	58.6 (17.4)
Size Type II	44.3 (9.8)	40.6 (6.1)	41.8 (11.3)	43.1 (10.1)	39.3 (8.5)	41.8 (10.5)
S.Factor I (x1/1000)	107.6 (59.1)	123.5 (31.9)	85.7 * (24.0)	150.5 (37.9)	114.5 (60.0)	181.9 (131.1)
S.Factor II (x1/1000)	64.0 (32.5)	47.5 (19.0)	65.6 (44.8)	50.1 (36.8)	55.7 (27.5)	53.5 (20.9)

Table 3-6. Mean values for muscle fiber characteristics on concave and convex sides of the vertebral column above, below and at the apex of the major curve in adolescent idiopathic scoliosis. (n=7; Size= μ m)
 (*=Significant difference found in equivalent muscles on opposite sides of the spine)

these fibers were randomly distributed and were not situated at any one site or depth more than any other. In one sample from the superficial group on the concave side below the apex, an isolated portion of the section demonstrated changes in type I and II muscle fibers. Very atrophic muscle fibers were distributed in a thin band or "stream" adjacent to fascicles of normal looking muscle. Although some rounding of fibers, fiber splitting, fiber phagocytosis and accumulations of connective tissue were occasionally apparent, these abnormalities were minimal and could not be associated with any particular site. Other abnormalities such as central nuclei, type grouping, target fibers, coil fibers and abnormally large fibers were not observed.

Moth-eaten type I muscle fibers (Dubowitz and Brooke, 1973) were observed in samples stained with NADH in five of the seven subjects, in both superficial and deep muscle groups. Although these fibers were observed to occur on the convex side of the curve to a greater degree and in both superficial and deep muscle groups, no consistent pattern could be established.

Perhaps most evident in Table 3-6 is the predominance in proportion and size of type I muscle fibers in all similar areas of both superficial and deep muscle groups. As well, a resultant strength factor component, utilizing proportion and size of muscle fiber, displayed a predominance of strength in type I fibers. This concentration in proportion, size and strength factor of

type I fibers consistently appeared on the convex side of the curvature. Contrarily, the situation was reversed for type II fibers.

Table 3-6 also shows that in some instances comparison of muscle fiber characteristics between concave and convex sides of the curvature produced differences sufficiently large to be statistically significant. In all cases the larger values were found to be on the convex side of the curve. In the superficial samples these significant differences were restricted to the percentage of type I fibers above and below the apex with the size of the type I fibers also being greater on the convex side but only below the apex. These differences above and below the apex of the curve combined to produce significant differences in the strength factor also. Although differences in percent and size of type I fibers were present at the apex, they were not sufficient by themselves to be significant but in the calculation of the strength factor their combined values were sufficient to produce a significant difference with the larger value again being found on the convex side. Therefore it would appear that in the superficial paravertebral muscles at least, there is a greater proportion of larger type I fibers on the convex side which combine to produce a greater force above, below and at the apex of the major curvature.

In the deep samples the only significant differences between the concave and convex values were found at the apex

of the curve; there was a greater percentage of type I fibers which were larger and which combined to give a greater strength factor on the convex side. Although other differences did exist in a pattern similar to that of the superficial muscles none of the differences was sufficiently large to be significant.

In Table 3-7 mean differences in muscle fiber characteristics between concave and convex sides of the major curve were similarly arranged according to site. It is of interest to note from the standard deviations, that relatively large variations existed in values for muscle fiber characteristics of equivalent muscles on opposite sides of the vertebral column in individual patients.

	ABOVE		APEX		BELOW	
	mean	sd	mean	sd	mean	sd
SUPERFICIAL						
% Type I	16.6	11.4	5.7	17.2	9.1	15.1
% Type II	-16.6	11.4	-5.7	17.2	-9.1	15.1
Size Type I	5.5	8.8	5.2	7.1	13.0	9.9
Size Type II	0.5	14.6	1.5	12.7	-2.9	5.3
S.Factor I*	55.3	56.5	38.9	28.1	101.8	76.6
S.Factor II*	-15.9	36.5	-6.0	34.3	-11.1	17.1
DEEP						
% Type I	5.0	15.1	14.0	11.4	5.0	11.0
% Type II	-5.0	15.1	-14.0	11.4	-5.0	11.0
Size Type I	2.8	11.7	7.9	5.8	8.3	12.0
Size Type II	-3.7	10.1	1.3	10.7	8.3	19.3
S.Factor I*	15.9	76.0	64.8	35.6	67.4	88.6
S.Factor II*	-16.5	24.5	-15.5	36.4	-2.3	34.9

Table 3-7. The mean differences in muscle fiber characteristics between concave and convex sides of the major curve in adolescent idiopathic scoliosis. (n=7)
 (*=strength factor $\times 1/1000$; Size= μm)

C. RHESUS MONKEY

The histological and histochemical techniques used in this study are used routinely to determine muscle fiber characteristics in human and animal. Muscle fiber outlines were sharp and clear and differentiation into types I and II was easily accomplished. A small number of moth-eaten, split, core and ringed fibers were observed in a few sections of mainly the deep musculature. These abnormalities were of random distribution without preference for any one site or vertebral level more than another. Other than the moth-eaten fibers, these abnormalities tended to be confined to one fascicle or part of the section and adjacent to fascicles with muscle cells of normal appearance.

Table 3-8 shows the mean values of the muscle fiber characteristics for each of the sites examined. It is apparent from the standard deviations that at any one site there are considerable ranges to be found in these characteristics between different subjects. However, most importantly, in spite of this, are the significant differences to be found in the muscle fiber characteristics at different levels of the vertebral column. There is a significant decrease in percentage of type I fibers at both superficial and deep levels as the vertebral column is descended (with a concomitant increase in percentage of type II fibers). This significant decrease in type I fibers is common to both sides of the column. A similar pattern (a decrease in value) although less consistent, is shown by

	(T3)		(T8)		(L3)	
	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT
	mean (sd)	mean (sd)	mean (sd)	mean (sd)	mean (sd)	mean (sd)
SUPERFICIAL						
% Type I	35 (8.7)	38 (6.5)	28 (4.4)	26 (5.8)	21 (3.6)	24 (3.3)
% Type II	65	62	72	74	79	76
Size Type I	44.4 (3.5)	43.2 (7.1)	41.8 (3.3)	41.5 (6.4)	41.3 (5.9)	40.4 (7.2)
Size Type II	51.4 (3.0)	51.8 (7.6)	49.5 (5.4)	49.5 (3.8)	49.1 (3.2)	46.1 (9.1)
S.Factor I (x1/1000)	54.4 (13.9)	56.6 (17.9)	38.6 (9.1)	33.9 (8.1)	28.8 (11.3)	31.6 (10.5)
S.Factor II (x1/1000)	133.4 (12.1)	130.7 (30.2)	138.8 (23.1)	144.8 (30.7)	150.5 (21.2)	130.1 (50.6)
DEEP						
% Type I	50 (6.6)	53 (7.8)	43 (9.3)	51 (9.7)	46 (10.4)	41 (6.1)
% Type II	50	47	57	49	54	59
Size Type I	44.7 (4.1)	41.2 (8.3)	42.0 (6.5)	46.0 (5.3)	43.0 (2.4)	38.3 (7.2)
Size Type II	50.0 (7.5)	42.6 (3.5)	43.5 (7.8)	48.3 (7.1)	*38.9 (4.4)	*47.8 (2.7)
S.Factor I (x1/1000)	79.0 (16.9)	74.0 (30.9)	61.1 (25.2)	83.5 (13.1)	67.5 (16.6)	36.8 (18.9)
S.Factor II (x1/1000)	101.9 (43.2)	66.1 (10.0)	86.4 (30.5)	93.1 (43.8)	*62.6 (10.2)	*106.7 (17.8)

Table 3-8. Mean values for muscle fiber characteristics on opposite sides of the vertebral column in adult female rhesus monkeys at levels: thoracic 3 and 8 (T3 & 8) and lumbar 3 (L3).

(N=5; Size= μ m)

Significant differences between equivalent muscles at different levels are too numerous to be shown here. They are discussed in the text.

*=Significant difference found in equivalent muscles on opposite sides of the spine

the size of the two types of fiber. These decreases in percentage and size of fiber type are further translated into decreases in strength factor as the column is descended although the increase in percentage of type II fibers restricts this decrease to involve only the type I fibers. It is interesting to note that this decrease in type I fiber characteristics is evident on both sides of the vertebral column, particularly when significant differences in fiber characteristics are not present between the two sides at any level (other than in size of type II fibers in the lower regions of the column).

Although Table 3-8 demonstrates that no significant differences exist between the two sides of the vertebral column (except in size and strength factor of type II fibers in one region on one side only) when values from several subjects are pooled together, this is not necessarily so for the individual. Table 3-9 shows the difference in mean values of muscle fiber characteristics between the two sides of the vertebral column. The standard deviations associated with these values demonstrate that large differences in muscle fiber characteristics can and do exist within individuals between the two sides of the vertebral column.

Table 3-10 shows the mean values for muscle fiber characteristics on opposite sides of the vertebral column in humans and monkeys. It is apparent that within the individual species there is little difference in almost all measurements between the two sides of the column. Exceptions

	(T3)		(T8)		(L3)	
	mean	sd	mean	sd	mean	sd
SUPERFICIAL						
% Type I & II	-2.8	6.3	2.4	3.3	-3.6	6.2
Size Type I	1.2	5.2	0.3	6.1	0.9	11.0
Size Type II	0.4	7.1	0.0	5.2	3.0	11.6
S.Factor I	-2.2	16.3	4.7	7.9	-2.9	20.0
(x1/1000)						
S.Factor II	2.7	29.1	-6.0	33.1	20.4	65.1
(x1/1000)						
DEEP						
% Type I & II	-3.2	3.0	-8.4	9.0	5.6	9.7
Size Type I	3.5	8.6	-4.0	9.7	4.7	7.1
Size Type II	7.4	8.4	-4.8	9.1	-8.9	2.7
S.Factor I	5.0	30.6	-22.4	21.5	30.8	25.1
(x1/1000)						
S.Factor II	35.8	38.4	-6.7	50.7	44.1	18.4
(x1/1000)						

Table 3-9. The mean differences of muscle fiber characteristics between corresponding muscles on the two sides of the vertebral column in rhesus monkeys. (N=5; Size= μ m)

	HUMAN L4-5		MONKEY L3	
	Left	Right	Left	Right
SUPERFICIAL				
% Type I	+61(18.8)	+50(13.5)	21(3.6)	24(3.3)
% Type II	*39(18.8)	*50(13.5)	79(3.6)	76(3.3)
Size Type I	#55.1(14.3)	\$62.2(9.3)	41.3(5.9)	40.4(7.2)
Size Type II	#36.3(10.7)	\$37.9(8.9)	49.1(3.2)	46.1(9.1)
S.Factor I	140.8(54.1)	158.8(67.0)	28.8(11.3)	31.6(10.5)
S.Factor II	44.7(40.8)	59.4(25.8)	150.5(21.2)	130.1(50.6)
DEEP				
% Type I	47(19.5)	55(14.0)	46(10.4)	41(6.1)
% Type II	53(19.5)	45(14.0)	54(10.4)	59(6.1)
Size Type I	&57.0(7.8)	57.6(15.0)	43.0(2.4)	38.3(7.2)
Size Type II	&41.2(11.1)	43.7(15.6)	X38.9(4.4)	X47.8(2.7)
S.Factor I	129.3(57.1)	150.6(81.7)	67.5(16.6)	36.8(18.9)
S.Factor II	82.2(63.2)	83.0(75.1)	x62.6(10.2)	x106.7(17.8)

Table 3-10. Mean values (S.D. in brackets) for muscle fiber characteristics on opposite sides of the vertebral column in humans and monkeys. Significant differences are indicated by similar symbols (+, *, #, \$, X, &, x). (Size= μ m; Strength Factor= $\times 1/1000$)

include a significant difference in percentage of type I fibers in the superficial muscles of the human (with the corresponding difference also found in percentage of type II) and the significant difference in size of type II fibers in the deep muscles of the monkey (which is also reflected in the corresponding strength factor). These isolated differences are difficult to explain.

In the human, the type I fibers are always significantly larger than the equivalent type II fibers at all sites. This is not the case in the monkey where the type II fibers are larger (though not significantly) in the superficial muscles and there is variation in the deeper layers.

A comparison between the two species (Table 3-11) reveals that there is a much greater, significant percentage of type I fibers in the superficial muscles of the human (with a consequent smaller percentage of type II fibers). This large difference (of approximately 30%) is not present in the deeper layers although there is still a significantly larger percentage of type I fibers in the deep layers of the human on the right side when compared with the monkey but this difference is considerably smaller than in the superficial layers (approximately 14%).

At all sites type I fibers were significantly larger in the human than in the equivalent muscle in the monkey (approximately 30% larger). The type II fibers were much the same size in the two species (average difference regardless

	LEFT		RIGHT	
	Monkey	Human	Monkey	Human
	L3	L4-5	L3	L4-5
	mean(sd)	mean(sd)	mean(sd)	mean(sd)
SUPERFICIAL				
% Type I	21(3.6)	* 61(18.8)	24(3.3)	* 50(13.5)
% Type II	79(3.6)	* 39(18.8)	76(3.3)	* 50(13.5)
Size Type I	41.3(5.9)	* 55.1(14.3)	40.4(7.2)	* 62.2(9.3)
Size Type II	49.1(3.2)	* 36.3(10.7)	46.1(9.1)	37.9(8.9)
S.Factor I	28.8(11.3)*	140.8(54.1)	31.6(10.5)*	158.8(67.0)
S.Factor II	150.5(21.2)*	44.7(40.8)	130.1(50.6)*	59.4(25.8)
DEEP				
% Type I	46(10.4)	47(19.5)	41(6.1)	* 55(14.0)
% Type II	54(10.4)	53(19.5)	59(6.1)	* 45(14.0)
Size Type I	43.0(2.4)	* 57.0(7.8)	38.3(7.2)	* 57.6(15.0)
Size Type II	38.9(4.4)	41.2(11.1)	47.8(2.7)	43.7(15.6)
S.Factor I	67.5(16.6)*	129.3(57.1)	36.8(18.9)*	150.6(81.7)
S.Factor II	62.6(10.2)	82.2(63.2)	106.7(17.8)	83.0(75.1)

Table 3-11. Mean values for paravertebral muscle fiber characteristics in humans and monkeys arranged to compare the samples from similar sites between the two species. Significant differences between sides are noted as *.

(Size= μ m; Strength Factor= $\times 1/1000$)

	HUMAN L4-5	MONKEY L3
	mean (sd)	mean (sd)
SUPERFICIAL		
% Type I	11.4(23.4)	-3.6(6.2)
% Type II	-11.4(23.4)	3.6(6.2)
Size Type I	-7.2(15.0)	0.9(11.0)
Size Type II	-1.7(7.2)	3.0(11.6)
S.Factor I	-18.0(68.8)	-2.9(20.0)
S.Factor II	-14.7(80.3)	20.4(65.1)
DEEP		
% Type I	-7.6(21.8)	5.6(9.7)
% Type II	7.6(21.8)	-5.6(9.7)
Size Type I	-0.7(15.3)	4.7(7.1)
Size Type II	-2.5(17.0)	-8.9(2.7)
S.Factor I	-21.3(97.8)	30.8(25.1)
S.Factor II	-0.7(83.9)	44.1(18.4)

Table 3-12. Mean differences of muscle fiber characteristics between left and right sides of the vertebral column in humans and monkeys.

(Size= μ m; Strength Factor= $\times 1/1000$)

of species approximately 14%) with no significant differences being found except in the superficial muscles on the left where the monkey fibers were significantly larger than the human. Neither species had consistently larger type II fibers at any of the biopsy sites.

Table 3-12 shows the mean differences of muscle fiber characteristics between both sides of the vertebral column in both the species studied. While these values might be considered relatively small, the comparatively large standard deviations associated with them demonstrate that in both humans and monkeys (perhaps slightly less in monkeys) a large difference in muscle fiber characteristics between the two sides of the vertebral column is to be expected at any one particular level. This is an interesting feature if it is felt that the function of the paravertebral muscles is to maintain a straight vertebral column. Apparently this may be achieved with muscular imbalance at any one particular vertebral level.

IV. DISCUSSION

The present work was undertaken to study the paravertebral muscle fiber characteristics in humans with disc disorders to assess its use as normal, humans with IS to assess abnormalities, if any, and normal rhesus monkeys to assess its potential as an animal model.

A. MUSCLE FROM LUMBAR DISC DISORDER

There is no simple way in which paravertebral muscle samples can be obtained from the supposedly healthy "normal" individual who is symptomatically free of back pain and stiffness. When using disc patients as a normal sample, apprehension exists as to the actual normality of the paravertebral musculature in the presence of pathology. But the results of this study have indicated that the muscle from these disc patients demonstrate for the most part normal characteristics and are considered to be the nearest as possible to normal.

As indicated, care was taken to ensure precise definition and reliability of muscle sampling from patients suffering from a disc protrusion. Other researchers (Polgar *et al.* 1973; Johnson *et al.* 1973; Fidler *et al.* 1975) confined their sampling to one side of the vertebral column, or were imprecise in their definition of the site of sampling. The importance of the site of sampling with its inherent variation has been stressed (Toman, 1979; McFadden

et al. 1981; Bagnall *et al.* 1983).

A large number of subjects were used and identical areas on both sides of the vertebral column were sampled. Furthermore, there was minimal delay between the taking and the freezing of the biopsy for sectioning in the cryostat, therefore lessening the possibility of shrinkage (Fidler *et al.* 1975). This has been a consideration because too long a time lapse may alter the histochemical characteristics of the tissue (Fidler *et al.* 1975). The histochemical analysis was performed to provide information not only on the general histological characteristics of the muscle and its fiber type composition but to determine whether there was any striking pathological change which had occurred due to the disc protrusion in otherwise normal individuals. In general, no obvious signs of pathology as described by Greenfield *et al.* (1957), Bethlem (1970), Dubowitz and Brooke (1973) and Fidler *et al.* (1975) were evident. There was apparent grouping of type II fibers in 3 patients. However, it is known that this feature occurs in apparently normal subjects and is indicative of natural recurrent cycles of motor unit denervation and reinnervation from a comparatively early stage in life (Johnson *et al.* 1973). None of these sections exhibited a "denervation" pattern as described by Dubowitz and Brooke (1973) and Dubowitz (1978) with uniform atrophy of the type I and II groups. In those cases where noticeably small muscle fibers were encountered it was of random distribution and appeared selective of type II fibers.

Jennekens *et al.* (1971, 1972) found changes in fiber type grouping with a loss of mosaic pattern but they attributed it to denervation and reinnervation associated with age. Selective type II grouping occurred in this study in subjects of 43, 52, and 60 years of age although the degree of grouping was not more evident with an increase in age. Jennekens *et al.* (1971, 1972) also observed the occurrence in old age of markedly atrophic fibers as well as a selection of structural changes including target fibers and pseudo-cores although Fidler *et al.* (1975) did not find these changes in multifidus muscle taken from patients with lumbar spinal derangement. The present work demonstrated the presence of central core and "moth-eaten" fibers occurring in type I fibers to a variable degree. However they were not associated with all ages as was found by Jennekens *et al.* (1971). The significance of these apparently abnormal fibers is difficult to assess and Dubowitz (1978) indicates that the reason for their occurrence is generally obscure and apparently normal. It might be expected therefore that a certain proportion of abnormal fibers are a normal occurrence in paravertebral musculature. This suggests that the disc protrusion has had little or no effect on the histological and histochemical appearance of the surrounding muscles with any apparent fiber abnormalities appearing to be a normal finding.

The establishment of a strength factor for each muscle introduced a method of combining the measurements of muscle

taken, into a functional concept. This was based on the findings of Haggmark *et al.* (1978) in which the paravertebral muscles are effective because of the forces they produce and these forces are dependent on the type of fiber and area involved. Haggmark *et al.* (1978) in studying vastus lateralis muscle using computed tomography, suggested that equivalent muscles have equal numbers of fibers. Therefore by combining measurements of fiber area and fiber type, an indication of force was introduced. This was necessary because comparison of isolated measurements might lead to erroneous conclusions; for example, a high percentage of small type I fibers might be opposed to a low percentage of large type I fibers on the opposite side of the column. Individually, the two percentages and the two sizes are very different but when combined, might balance with each other. The idea of a strength factor has been used in other studies (Yarom and Robin, 1979) and is based on the concept that the strength of a muscle is proportional to its cross-sectional area. As it is also based on the percentage of a particular fiber type and not the absolute number of fibers, it is only of use when comparing muscles with equal numbers of fibers, as might be found in equivalent muscles on both sides of the body. It should also be noted that the units of the strength factor for type I fibers might not be equivalent to those for type II fibers and although large differences between the two types might exist, no valid comparisons can be made. The strength factor serves as an

indicator of the potential force within a muscle of a particular type of fiber and is useful for comparison with equivalent muscles on the opposite side of the body (Bagnall *et al.* 1983).

Affected versus Unaffected Sides

In the presence of spinal pathology questions regarding the normality of surrounding musculature can be raised but the results fail to demonstrate any differences in muscle fiber characteristics that can be related to the side of the protrusion (Tables 3-1 and 3-2). Moreover the lesions were evenly distributed, occurring to the left in 9 cases and to the right in 9. This suggests that any variation found in the muscle characteristics is inherent within the muscle and not related to the disc protrusion. The large size of this variation in muscle characteristics warrants further investigation.

The mean values calculated for the strength factor were similar between affected and non-affected sides of the vertebral column but again the actual differences between the two sides of the vertebral column in individual cases are concealed. Large individual differences did occur but it is important to emphasize that they do not appear to be related to the side of the disc protrusion.

It is known that type I fibers participate mainly in sustained tonic activity. The results in Table 3-1 are suggestive of a more postural than rapid, phasic function in

the musculature of this area and highlights the importance of the type I fibers in maintaining an erect vertebral column. It is of further interest to note the predominance of "strength" in the type I tonic or slow muscle group. It could be thought that these fibers, predominant at the base of the vertebral column, have increased in size to meet the requirements of "protective splinting" in an area of pain, associated with a disc protrusion. This predominance however appears to exist quite normally as evidenced by similar investigations (Polgar *et al.* 1973; Johnson *et al.* 1973) using lumbar muscle from autopsy specimens. Therefore it is suggested that the muscle characteristics presented in Table 3-1 represent normal lumbar paravertebral muscle for the results compare well with other studies.

It is quite possible that the disc protrusion has affected the muscles on both sides of the vertebral column. Such a bilateral effect would not be evident from our analysis. However, this is not a major consideration as our basic measurements seem to accord fairly well as far as valid comparisons can be made, with the results of other studies which used biopsy material from autopsy specimens of patients who were possibly free of symptoms related to spinal disorder (Johnson *et al.* 1973; Polgar *et al.* 1973; Fidler *et al.* 1975). The difficulty for comparison between studies exists because of the different sites used in obtaining the paravertebral muscle samples. See Tables 4-1 and 4-2.

This analysis, Table 3-1, suggests that paravertebral muscle is unaffected by an acute disc protrusion under the conditions outlined. The muscle from such patients may be considered as typical of normal paravertebral musculature when comparison is made with other muscle. It is not suggested that long-standing disc protrusions could not eventually have some permanent effect on the paravertebral musculature but that under the conditions outlined the disc protrusion does not affect the surrounding musculature. It is of particular importance that the muscular measurements taken in this study relate directly to the measurements that are being used currently to investigate pathological muscle and related areas such as IS (Fidler *et al.* 1974; Fidler and Jowett, 1976; Yarom and Robin, 1979).

Left versus Right Sides

The mean values reported in Table 3-3 compare fairly well with those of other workers (See Tables 4-1 and 4-2) with the exception of type II fiber diameters. It is difficult to make exact comparisons because these results are more clearly defined and less general. The range of values for normal in this study are sometimes larger than those of other workers but this may be due to the large number of subjects and also to the more specific method of muscle sampling.

The mean value for diameters of type II fibers are smaller for both superficial and deep muscles when compared

with other work, although the actual values are not significantly different. It could be thought that the disc protrusion might have affected the muscle on both sides of the vertebral column resulting perhaps in smaller fibers through inactivity of the type II fibers which are used for powerful contractions related to voluntary activity (Sivachelvan and Davies, 1981). The method of analysis would not reveal such changes and this requires further investigation perhaps using muscle samples obtained from trauma patients. Other variables, such as site of sampling must also be considered as having a large influence on these measurements, with other workers perhaps taking their samples from other vertebral areas. Although information is scant on the human it has been demonstrated that paravertebral muscle fiber characteristics vary according to the vertebral level sampled in the monkey and Spencer and Zorab (1976) found variations in the rabbit. It is reasonable to presume that this variation in muscle makeup would be found in humans according to vertebral level.

Perhaps of greatest significance are the large standard deviations found to be associated with the mean differences between the left and right sides of the vertebral column (see Table 3-4). These standard deviations indicate that large differences in muscle fiber characteristics can exist between the two sides of the vertebral column. Any thoughts of a balance between the muscle characteristics of the two sides of the column at one level would appear to be false.

SOURCE	%Type I fibers			Size Type I fibers			Size Type II fibers		
	lower upper			lower upper			lower upper		
	limit	mean	limit	limit	mean	limit	limit	mean	limit
A	23.4	61.0	98.6	26.5	55.1	83.7	14.9	36.3	57.7
B	33.3	58.4	83.5	41.0	60.0	78.0	32.0	57.0	83.0
C	50.0	62.1	75.0	42.3	68.7	88.6	45.5	65.7	91.4

Table 4-1. A comparison of the available data for
the SUPERFICIAL muscles of the back.
(Size = μ m)

A = Results from this study - left side only.
B = Johnson *et al.* (1973) and Polgar *et al.* (1973).
C = Sulemana and Suchenwirth (1972)

SOURCE	% Type I fibers			Size Type I fibers			Size Type II fibers		
	lower		upper	lower		upper	lower		upper
	limit	mean	limit	limit	mean	limit	limit	mean	limit

A	8.1	47	86.1	41.4	57.0	72.6	19.0	41.2	63.4
B	32.0	54.9	77.8	49.0	61.6	73.0	34.0	53.6	74.0
C	40.5	66.6	92.5	53.2	73.4	89.2	25.9	59.6	80.2

Table 4-2. A comparison of the available data
for the DEEP muscles of the back.
(* calculated from area data in their
paper and assuming circular fibers)
(Size = μm)

- A = This paper - left side only
- B = Johnson *et al.* (1973) and Polgar *et al.* (1973)
- C = Fidler *et al.* (1975)

As fiber type proportion is genetically determined (Stickland, 1981) and neuronally controlled (Fischman, 1972) it is resistant to change and it is conceivable that the difference in proportions might be related to handedness (Sahlstrand, 1981). On the other hand, hyperplasia may be attributed to longitudinal splitting (Hall-Craggs, 1970), transformation (Hoyle, 1983), or new fiber production following trauma (James, 1976; Nag and Foster, 1981) in view of the fact that many lumbar disc problems become apparent although without an awareness by the patient of some initial injury. Unlike IS, these imbalances in muscle fiber proportion are not consistently situated on one side of the vertebral column and the column remains straight.

Fiber size can be altered through exercise and activity (Saltin *et al.* 1977). It might be thought that changes in fiber size could occur to compensate for different proportions of fiber types by developing equivalent potential forces which would maintain a balanced erect column, but the large differences in strength factor do not support this. It would appear that at any one level of the vertebral column large differences in muscle fiber characteristics can exist. This is not to say that the combination of these characteristics from various related areas of the column might not lead to a total, overall balance. In this respect it is interesting to note that the mean values of muscle characteristics (Table 3-3) are similar when comparing opposite sides of the vertebral

column. If the range of variation is consistent for all vertebral levels then within one individual the sum total of muscle characteristics on one side of the vertebral column might well balance those on the other side. Analysis of muscle samples from all levels of the vertebral column are required to examine this.

It is difficult to offer an explanation for the significantly greater proportion of type I fibers on the left side in the superficial muscle (and the converse; greater proportion of type II on the right side). Sample bias does not play a part as 9 subjects had disc protrusions to the right, 9 to the left and 1 was central. If the potential force of a muscle is considered as a combination of the fiber type complement and the fiber area (strength factor), it is worthy to note that no compensatory change in size of fiber type has occurred on the right side of the column. This might have been expected if a balance was thought to exist between the muscle fiber characteristics on the two sides of the vertebral column.

Separation of the data into different age groups revealed that the muscle fiber characteristics did not change as the subjects got older. While this may be unexpected for other muscle groups whose activity level might be less with age it may not be so for the paravertebral muscles, for their function is to maintain an erect vertebral column, a task which does not diminish with increasing age. This is in contrast to the work of Larsson

et al. (1978) who found significant changes in characteristics when studying muscle samples from VL of male subjects aged 22 to 65 years. Recently there have been some questions raised (Bagnall *et al.* 1983; McFadden *et al.* 1981) by sampling techniques used for VL as it is known that vastus intermedius (VI) may be inadvertently sampled instead and this could have some effect on results obtained. Definition of sampling site is rarely well defined and age changes in equivalent muscles on both sides of the body appear not to have been considered.

Analysis of the data based on sex also showed little difference in paravertebral muscle characteristics between male and female, the only significant difference being between the size of type II fibers in both superficial and deep groups on the left side (see Table 3-5). This was extended to the strength factor also in the deep group only. Basically the size of type II fibers for the women were always smaller than those for the men and those on the left side appeared to be particularly small. These results compare well with those of Brooke and Kaiser (1970) mentioned earlier. Type II fibers are considered to be phasic being responsive to non-reflex activities and again this small size of fibers might be related to handedness (Sahlstrand, 1981). It is perhaps important to note that a similar situation does not exist for the type I fibers which are similar in size in the female to the males. Costill *et al.* (1976a) found that male track athletes possessed

significantly larger type I and II fibers than female athletes but stated that caution must be exerted when comparing subjects as it is impossible to control the degree of fiber shortening at the time of sampling and possibility of anabolic steroid ingestion.

The typical standards presented in Tables 3-3 and 3-5 can now be used in studies involving abnormal paravertebral muscle. Such detailed standards were not previously available. More studies are required to expand this data to include other vertebral levels, increased age ranges of subjects and comparison with muscle samples from trauma and/or autopsy specimens.

B. MUSCLE FROM ADOLESCENT IDIOPATHIC SCOLIOSIS

To examine histologically and histochemically the characteristics of the paravertebral muscles surrounding the curve in IS a specific method for obtaining the samples has been described and both superficial and deep muscles have been included in the analysis. Furthermore a comprehensive analysis of the paravertebral muscles associated with the complete curvature has been made by including samples taken from above and below as well as at the apex of the major curvature.

Spencer and Zorab (1976) examined 6 muscle biopsy specimens from 3 sites (above, below and at the level of the apex of the primary curvature) from the deep surface of the erector spinae mass in 35 adolescents (mean age 13.8 yrs)

who were suffering from IS. However, they did not precisely define the sites of sampling and this has been shown to be of considerable importance (Toman, 1979; Bagnall *et al.* 1983). They did, however, find many abnormalities in their muscle specimens including internal nuclei, unusually large (200 μ m) and small (5 μ m) fibers, absence of type II fibers, whorling, target fibers, coil fibers and grouping. These abnormalities were not related to any particular site nor were they found more commonly on one side more than the other. These results are not inconsistent with the results of this study although the incidence of abnormal fibers may be slightly higher in the results presented by Spencer and Zorab (1976). It appears important that in so-called "normal" paravertebral muscle (obtained from patients suffering from acute spinal dysfunction) there is a relatively high incidence of abnormality in muscle fiber characteristics (Dubowitz and Brooke, 1973). Dubowitz and Brooke (1973) estimate that normal muscle may have up to 10% abnormal fibers and yet be considered as acceptable of normal. The significance and means of production of these abnormalities has yet to be established.

Comparison of our data with that of other studies (Fidler *et al.* 1974, 1976; Hoppenfeld, 1974; Spencer and Eccles, 1976; Spencer and Zorab, 1976; Yarom and Robin, 1979; Green, 1981; Sahgal *et al.* 1983) is difficult because of variations in sampling sites and expression of size (area or diameter). Furthermore, our results are more

comprehensive because we have included both superficial and deep layers of muscle. Similar data to ourselves was collected by Yarom and Robin (1979) although they only sampled the "deep layer of the erector spinae" muscle with possible inclusion of the transversospinal layer. Fidler *et al.* (1974, 1976), Spencer and Zorab (1976), Yarom and Robin (1979) and Green (1981) found a predominance of type I muscle fibers in the deep spinal musculature but only on the convex side at the apex of the curvature. It is reassuring that our results accord very closely with the findings of other workers but also extend the differences to the superficial muscles with the finding that there is a significant dominance of type I muscle fiber characteristics at all levels on the convex side of the curvature. These findings are supported by the EMG results of Riddle and Roaf (1955) and Zuk (1962) in which greater electrical activity was found in the musculature on the side of the convexity. Fidler *et al.* (1974) suggest that increased electrical activity reflects greater tonicity and therefore are representative of more type I or tonic, slow muscles which are used to maintain posture (Sivachelvan and Davies, 1981). Therefore, it could be anticipated that multifidus with its more sustained tonic action should shorten on the side of the convexity. This shortening of multifidus on the side of the convexity is consistent with the concept that muscle imbalance produces a deformity in which the more tonically active muscle contracts and becomes shorter. If scoliosis

were produced by some other mechanism, atrophy of type I fibers should occur (Engel *et al.* 1966) in the passively shortened multifidus. Yarom and Robin (1979) demonstrated type I atrophy but noted these changes on the side of the concavity and it is not clear as to the specific muscle sampled. Contrary to the results of Engel *et al.* (1966) and Yarom and Robin (1979) generalized atrophy of type I muscle fibers was not demonstrated in this study.

With these factors in mind, one could question the validity of delivering faradic muscle stimulation to the paravertebral muscles on the side of the convexity (Bobechko, 1979). If the intention of muscle stimulation is to create a balance between type I and II muscle fibers on opposing sides of the column, questions must be addressed as to which fiber type and which side. Subsequent modification of pulse duration and rest period must be considered in order that the treatment is both effective and therapeutically correct. Since type II muscle fibers are smaller in the human and more susceptible to atrophy than type I fibers (Dubowitz and Brooke, 1973) then possibly the type II muscle fibers would require stimulation.

Fidler and Jowett (1976) present a mechanical hypothesis in which a single, deep muscle imbalance can conceivably create a scoliosis with the stronger muscle being surprisingly on the convex side at the apex of the curvature. Our results lend support to this hypothesis. If the isolated deep muscle imbalance has resulted in

development of scoliosis, then it is possible that the long, superficial paravertebral muscles would resist this development by increasing the dominance of the type I postural fibers on the convex side of the curve in an effort to retain alignment of the pelvic and pectoral girdles. The results of this study support this extension of the hypothesis with a dominance of type I fiber characteristics being found at all levels on the convex side of the curvature. It is unfortunate that the dominance is represented above and below the apex of the curvature by an increase in percentage of type I fibers, for this is a characteristic that appears to be genetically controlled (Stickland, 1981) and constant throughout life. As such, this characteristic is probably unable to develop secondarily to the scoliosis although it is conceivable that during the growth spurts a disproportion of fiber types is produced. This information is not available at present but is being considered for future research endeavors. However, the type I fibers are larger on the convex side of the curve (significant below the apex) and this could well be a product of the original curve. Conversely, the dominance of superficial type I fiber characteristics on the convex side of the curvature might contribute to the primary cause of the scoliosis although it would be difficult to include the superficial muscles in the application of the hypothesis of Fidler and Jowett (1976).

Khosla *et al.* (1980) found sarcolemmal defects in multifidus muscle at the myotendinous junctions. Being that the faulty junctions were found in IS at the apex on the concave side of the primary curve, one could speculate that the resultant increase in strength factor of type I muscle fibers on the convex side could exert an overpowering sustained force against an unequally opposed or "injured" concave multifidus. This imbalance in relative strengths of the deep muscles on opposite sides of the spine might well be a primary inherent deforming factor with resultant shortening of the convex multifidus and atrophy of type II fibers. Assuming that fiber type proportions are established at this age, the sarcolemmal defects in concave multifidus might be indicative of secondary trauma, due to the unyielding primary deforming force of the convex multifidus. Although to date the author has not done EM studies on the paravertebral muscles in IS, this project will be undertaken in the future.

The results of this study support the findings of other workers in the study of human scoliosis and extend the results to include a survey of all the muscle surrounding the curve, including both superficial and deep layers. A complex picture of muscle involvement emerges in which it is difficult to distinguish cause from symptom. It is suggested that the underlying cause of the curve might be the imbalance in the deep muscles at the apex of the curve (supporting the hypothesis of Fidler and Jowett, 1976) and

that the superficial muscle imbalance is a symptom. Some of these 'secondary' changes in the superficial muscles, although significant, are thought to be genetically determined and this suggests once more that IS is the result of the unfortunate interaction of a multitude of possibly normal differences.

C. MUSCLE FROM RHESUS MONKEY

The vertebral column might be considered as being basically most stable in the lower lumbar region where it is firmly attached to the pelvic girdle and is supported on either side by large muscle masses of the erector spinae and adjacent lumbar fascia. Higher regions, such as thoracic, might be considered less stable as they are further from the firm foundation provided by the pelvic girdle. Furthermore, the splinting effect of the columns of erector spinae and accompanying fascia is more dissipated in the thoracic region as the muscle in particular becomes smaller. If this description of stability of the vertebral column is valid then it is not difficult to appreciate the need for greater postural tone in the paravertebral muscles as the column is ascended. Postural tone is provided by the type I fibers (Sivachelvan and Davies, 1981) and this would manifest itself by an increase in percentage of type I fibers. This would explain the major findings of this study regarding the rhesus monkey. It is difficult to be entirely conclusive because the change in percentage of fiber types could be due

to several causes independent of changes in absolute number. An absolute increase in number of type I fibers, an absolute decrease in number of type II fibers, or a relative increase in number of type I fibers would all be manifested as an increase in percentage of type I fibers. As the total number of fibers in each muscle was not counted, the reason for the increase in percentage of type I fibers is not known. All that can be accurately stated is that paravertebral muscle fiber characteristics of the monkey are dependent on vertebral level and this is most manifest in the percentage of fiber types. Perhaps it is significant that Spencer and Zorab (1976) when studying muscle at various vertebral levels in normal and scoliotic rabbits, also found a high percentage of type I fibers in the cervical and upper thoracic regions.

It is perhaps more difficult to assess the relevance of these results to human paravertebral musculature. The rhesus monkey is often used in laboratory studies because of its similarity to the human. With regard to muscle arrangement and bony structure this is probably true. Most monkeys, as opposed to apes, are really quadrupeds, travelling on all four limbs needing only to move their front limbs backward and forward with small amounts of sideways motion (Eimerl and DeVore, 1979). With regard to posture, and consequently postural control, it is almost certain that differences between monkeys and humans will exist although the extent of this in relation to fiber characteristics of paravertebral

muscles is unknown.

Various conclusions can be made following comparison of the vertebral muscle fiber characteristics of the human and monkey. Qualitatively, the muscle samples are similar with clearly defined polygonal cell boundaries that are easy to recognise using routine staining procedures. The presence of a small percentage of abnormal fibers (Dubowitz and Brooke, 1973) in both species is of particular importance as this suggests that these are truly representative of typical paravertebral muscle and are probably present in most samples of even the most "normal" muscle. The reason for their presence and their function remains unknown but it is becoming clear with their constant appearance that they form a small part of the paravertebral muscle structure. Perhaps more attention needs to be given to them than has been given in the past.

While there is considerable similarity between the two species in qualitative terms, there are differences when examined quantitatively. The superficial paravertebral muscle of the human is vastly different to that of the monkey with almost all of the paravertebral muscle fiber characteristics observed at similar sites being significantly different. In the human there is a greater proportion of type I fibers which are significantly larger than those in the monkey. Conversely there is a smaller proportion of type II fibers in the human which curiously, are smaller than in the monkey. These differences might

reflect differences in function of these paravertebral muscles between the two species.

With regard to posture, the monkey has virtually a horizontal spine, while the human attempts to maintain an erect column. Postural tone and balance therefore may be of more importance to the human and it is perhaps of no surprise to find present a greater proportion of postural fibers (Sivachelvan and Davies, 1981) in the paravertebral muscle. Conversely, if the superficial muscles with their widely separated attachments are considered as the primary erectors of the vertebral column it is no surprise to establish an emphasis of large type II fibers in the monkey whose main concern is with flexibility and movement rather than erect stability. This would suggest that the paravertebral muscle of the human while being similar in gross appearance to the monkey has significantly different fiber characteristics imposed by the functional demands of maintaining a more erect column. Extrapolation of the results of this study to the human must therefore be made with considerable caution, although it would seem reasonable to suggest that, as with the monkey, paravertebral muscle fiber characteristics are dependent on the vertebral level being studied.

Differences found in the deep musculature are more difficult to explain. The muscles at this level have much closer attachments than those in the superficial layers and presumably are more concerned with maintenance of the

relationships between consecutive vertebrae and groups of vertebrae. In this respect it is not surprising to find fewer differences between the two species as the integrity of the relationship between adjacent vertebrae is common to both species. The major differences are to be found in the superficial layers of muscle which control the major arrangement of the vertebral column.

Assessment of the suitability of the rhesus monkey as a model for the production of IS on the basis of its paravertebral muscle fiber characteristics is therefore difficult. Those studies that have investigated the paravertebral muscle surrounding the primary curvature in idiopathic scoliosis (Fidler *et al.* 1974; Spencer and Zorab, 1976; Fidler and Jowett, 1976; Yarom and Robin, 1979, 1979b) have suggested that an imbalance of muscle fiber characteristics exists in the deep layer of muscles at one particular vertebral level. In this respect it is encouraging to note that there are considerable similarities between the muscle fiber characteristics of the deep layers of the monkey and the human. However the integrity of the whole vertebral column is the summation of many contributing factors all of which play a significant part. The major differences present in the superficial layers of paravertebral muscle are discouraging in that it is their effects that apparently have most influence on the column as a whole because of their widely separated attachments. It would appear that the erect vertebral column of the human

and its maintenance by the paravertebral musculature present a considerable obstacle when attempting to develop an animal model for the study of vertebral mal-development. This study attempted to provide these facts and compared the fiber characteristics of human and monkey paravertebral muscle in the lower lumbar region.

D. SUMMARY AND CONCLUSIONS

Despite extensive investigation, the cause of IS remains obscure although muscle has been implicated. Preliminary studies have suggested that an imbalance in fiber type proportions exists at the apex of the major curve by way of a greater percentage of type I fibers being present on the convex side of the curve. However, few studies have dealt specifically with the histological and histochemical analysis of equivalent superficial and deep muscles on both sides of the vertebral column at the same specific predetermined sites either in the normal individual or in the individual with IS. As a result, normal standards for paravertebral muscle fiber characteristics at various depths and levels of the vertebral column are not available for determination as to whether or not the differences found in IS are in fact normal features. It is also not known how these characteristics change with age and gender within the same muscle at the same level of the vertebral column.

The aim of the study was to obtain "normal" standards for human paravertebral muscle fiber characteristics and to

determine the differences, if any, in muscle fiber characteristics associated with scoliosis. In addition, analysis of muscle biopsy samples taken at various vertebral levels from rhesus monkeys was done for comparison of muscle fiber characteristics with those of the human as significant similarities between the two species would enable the use of the monkey as an animal model.

The results not only establish standards for typical human paravertebral muscle in the lumbar region but support and extend the findings of other workers. A significantly larger percentage of type I fibers was found in multifidus at the apex of the curve on the convex side as well as in the superficial muscles above and below the apex on the convex side, presenting a complex picture of muscle fiber characteristics associated with idiopathic scoliosis.

The rhesus monkey demonstrated muscle fiber characteristics in the superficial paravertebral group which were vastly different to that of the human, being significantly different at almost all similar sites. Fewer differences were found in the deep group between the two species reflecting possible correlation for study as an animal model. Most apparent were significant differences to be found in percentage and size of muscle fibers in both superficial and deep muscle groups between the vertebral levels. The findings indicate that the rhesus monkey, although not ideal, has considerable similarities to the human and warrants consideration as an animal model for the

study of scoliosis.

Several unanswered questions encourage the investigator to approach future projects involving the histological, histochemical and ultrastructural analysis of typical human paravertebral muscle at all vertebral levels, from individuals exhibiting straight spines such as trauma patients. Perhaps if a complete profile could be established for "normal" paravertebral muscle, taking into consideration level, age and gender, valid comparisons could more readily be made in pathological situations. Further endeavors will not only include an enlargement of population sample but also additional pertinent patient history and data.



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APPENDIX A

GROSS MORPHOLOGY OF HUMAN PARAVERTEBRAL MUSCLES

Attachments will be designated, for convenience as *origin* and *insertion* referring to "least moveable" and "most moveable" attachment respectively.

Erector Spinae Aponeurosis (ESA): arises from the medial aspect of the dorsal portion of the iliac crest, the sacral and lumbar spinous processes and supraspinous ligaments.

Lumbar Intermuscular Aponeurosis (LIA) (Bogduk, 1980): with attachment to a linear area on the medial aspect, dorsal segment of the iliac crest just rostral to the posterior superior iliac spine. Fans out, extending into the lower half of the dorsal lumbar region, terminating as fleshy muscle fibers on its ventral edge but separated from lumbar transverse processes 1-4 at about their middle third by a fat-filled space. Continuous dorsally with the erector spinae aponeurosis but runs in a somewhat parasagittal direction. See Plate H-9.

The following table is a compilation of paravertebral muscle attachments from various major sources (Anson, 1966; Gray, 1973; Hollinshead, 1974; Last, 1978; Hollinshead and Jenkins, 1981; and Woodburne, 1983). Hollinshead (1974) states "There is, perhaps, no entirely satisfactory grouping of the muscles of the back, but a reasonably successful one can be based upon the general direction of the muscle

bundles and their approximate lengths" and "all except the shortest and deepest-lying muscles of the back have multiple origins and insertions, arising from a number of consecutive vertebrae or ribs, and inserting above into a number of consecutive vertebrae or ribs". Therefore, although there are differing descriptions and group classifications for the various layers, they are minor. Comparisons by author were not regarded as necessary to this study although Chapter 1 can be consulted for a brief discussion on the differences in nomenclature.

HUMAN PARAVERTEBRAL MUSCLE ATTACHMENTS

C,T,L,S = cervical, thoracic, lumbar, sacral vertebrae

TP = transverse process(es); SP = spinous process(es)

ArP = articular process(es); AcP = accessory process(es)

MaP = mamillary process(es); ESA = erector spinae

aponeurosis; LIA = lumbar intermuscular aponeurosis

SUPERFICIAL - Lateral to Medial

MUSCLE	ORIGIN	INSERTION
ILIOCOSTALIS		
-lumborum	ESA; dorsal segment iliac crest; lateral surface LIA	Lateral third L1-4 TP; angles of lower 6 ribs
-thoracis	Lower 6 ribs	Upper 6 ribs; post tubercle C7 TP
-cervicis	Ribs 3-6	Posterior tubercle C4-6 TP
LONGISSIMUS		
-lumborum	ESA; dorsal segment iliac crest; medial surface LIA	All lumbar AcP; all length L5 TP; medial third L1-4 TP
-thoracis	ESA	Lower 11 ribs; adjacent margin TP
-cervicis	T1-4(5) TP	Posterior tubercle C2-6 TP
-capitis	T1-4(5) TP; C4(5) ArP	Dorsal margin mastoid process
SPINALIS/SEMI SPINALIS		
-thoracis	T6-10TP	C6-7, T1-4SP

-cervicis	T1-5(6)TP	C2-5SP
-capitis	T1-6, C7TP; C4-6 ArP; Occasionally C7, T1SP	Occipital bone between superior and inferior nuchal lines.

MULTIFIDUS - present in all regions of the spinal column
however largest in the lumbar region

ESA; dorsal sacrum and Sacroiliac ligaments; lumbar MaP; thoracic TP; C4-7 ArP	SP entire length of spine, spanning from 1 to 4 vertebrae
--	---

ROTATORES - present throughout the column however
best developed in the thoracic region

Upper and posterior part TP of one vertebra	Lower border, lat surface lamina, one vertebra above
---	--

INTERSPINALES - in pairs; largest in the cervical and
lumbar regions

-cervical	C2SP to T1SP between adjoining vertebrae
-thoracic	T1SP to T2(3)SP between adjoining vertebrae and lacking in the mid-thoracic region
-lumbar	L1SP to L5SP between adjoining vertebrae; T12SP to L5SP occasionally

INTERTRANSVERSarii

-cervical	Best developed; C1TP to T1TP; ventral and dorsal slips; dorsal slip with medial and lateral divisions
-thoracic	T9TP to L1TP between adjoining vertebrae
-lumbar	Mediales: AcP lumbar vertebrae to MaP lumbar vertebrae Laterales: Ventral portion lumbar TP TO TP Dorsal portion lumbar AcP TO TP

APPENDIX B

MUSCLE FIBER STAIN TECHNIQUES

TOLUIDINE BLUE (Chayen et al., 1973)

1. Stain in 1% toluidine blue in 1% borax for 30 seconds.
2. Wash in distilled water.
3. Dehydrate in ascending alcohols.
4. Clear in xylene and mount in D.P.X..

This stain was used as a quick method for determining whether the section of the block being cut at that time was satisfactory for further stain processing by subsequent methods. This eliminated time and effort by ensuring that the sections had an adequate number of muscle cells present. In certain blocks an accumulation of fat and fascia were present at some levels.

MASSON'S TRICHOME METHOD (Masson, 1929; Modification of Armed Forces Institute of Pathology Manual, Third edition, 1968)

FIXATION: Bouin's or 10% formalin. Mordant frozen sections in Bouin's fluid for 1 hour at 56° or overnight at room temperature.

TECHNIQUE: Paraffin or frozen sections at 10 μ m.

STAINING PROCEDURE

1. Wash in tap water until yellow colour disappears and rinse in distilled water.
2. Stain 10 minutes in Weigert's hematoxylin.
3. Wash well in tap water (10 minutes).
4. Rinse in distilled water.
5. Stain for 1 minute with Beibrich scarlet-acid fuchsin (solution should be half the strength of the original recipe).*
6. Rinse in distilled water.
7. Differentiate in 5% phosphotungstic acid solution for 15 minutes and discard solution.
8. Stain in Light Green solution for 1 minute.*
9. Rinse in distilled water.
10. Immerse in glacial acetic solution for 3 minutes.
11. Dehydrate in 95% alcohol, absolute alcohol, clear and mount.

* Times for staining are dependent upon initial test runs to determine degree of staining desired. In this study

the length of time found necessary for satisfactory definition of cell and intercellular detail was 2-21/2 minutes as Celloidin coating was used to prevent floating away of sections during the staining process.

SOLUTIONS:

Bouin's Solution

Picric acid saturated aqueous soln	750ml
Formalin, 37-40%	250ml
Glacial acetic acid	50ml

Weigert's Hematoxylin

Sol. A: Hematoxylin crystals	1gm
Alcohol, 95%	100ml
Sol. B: Ferric chloride, aqueous 29%	4ml
Distilled water	95ml
Hydrochloric acid, conc	1ml

Mix equal parts of Sol. A and B

Beibrich Scarlet-Acid Fuchsin

Beibrich scarlet, aqueous 1%	180ml
Acid fuchsin, aqueous 1%	20ml
Glacial acetic acid	1ml

Phosphotungstic Acid

Phosphotungstic acid	5gm
Distilled water	100ml

Light Green 2%

Light green SF yellowish	2gm
Distilled water	98ml
Glacial acetic acid	1ml

Celloidin Solution

Working solution:

Celloidin	200ml
Ether	500ml
Absolute alcohol	500ml

Take slides through xylene to absolute alcohol and dip 2-3 times in celloidin solution. Air dry between dips (3-4 minutes) but do not allow to dry completely.

RESULTS:

Nuclei.....	black
Cytoplasm, muscle	red
Collagen, mucous.....	green

NADH-Tetrazolium Reductase: DPNH Diaphorase (Dubowitz and Brooke, 1973)

1. Incubate tissue for 30 minutes at 37° Celsius in the following solution:

0.2 M-Tris buffer (pH 7.4)* 30ml

NADH (reduced diphosphopyridine

nucleotide 24mg

NBT (Nitro blue tetrazolium) 30mg

2. Adjust pH of solution to 7.4.
3. Rinse in distilled water.
4. Mount in glycerin jelly.

*Tris Buffer**

Tris crystals 24.2g

Distilled water 1000ml

RESULTS:

Type I muscle fibers - dark blue

Type II muscle fibers - light blue

ADENOSINE TRIPHOSPHATASE (Guth, L. and Samaha, F.J., 1970, Experimental Neurology, 28, p. 365-367)

TECHNIQUE

Demonstration of Alkali-Stable ATPase.

Type I - light; Type II - dark, brown-black

1. Fix sections in Solution 1 for 5 minutes.
This step is optional and not required for human material.
2. Rinse slides in Solution 2 for 1 minute with agitation and drain excess solution on blotting paper.
3. Preincubate in Solution 3 for 15 minutes.
4. Rinse slides in Solution 2 (two changes, 1 minute each) and drain excess solution.
5. Incubate for 15-60 minutes in Solution 4 at 37° Celsius. Solution 4 is filtered into a staining jar that is prewarmed to 60° Celsius as this rapidly warms the solution to about 37° Celsius.
6. Wash in three 30 second changes of Solution 5 and drain excess solution.
7. Place in Solution 6 for 3 minutes.
8. Wash in four 30 second changes of Solution 7 and drain excess solution.
9. Place in Solution 8 for 3 minutes.

10. Wash in running tap water for 3-5 minutes, dehydrate in graded alcohol, clear in xylene and mount.

Demonstration of Acid-Stable ATPase

Type I - dark, brown-black; Type II - light

1. Preincubate the unfixed sections in Solution 9 for 5-30 minutes and drain excess solution.

Complete the preparation as per steps 4-10 above.

Do not incubate slides that have been preincubated in acid or alkali in the same jar of incubation solution.

SOLUTIONS:

Quantities given are for one 50ml Coplin jar.

1. *Fixative* (5% formalin buffered

at pH 7.6)

Formaldehyde soln (40%)	50ml
Na cacodylate (MW 160)	31g
CaCl ₂ (MW 147)	10g
Sucrose (MW 342)	115g

Bring to final volume of 1 liter with water.

2. *Rinse Solution* (18mM CaCl₂ in

100mM Tris(hydroxymethyl aminomethane) pH 7.8)

Tris (MW 121)	1.21g
CaCl ₂ (0.18 M)	10ml
Distilled water	90ml

Adjust pH to 7.8 with HCl (1-6N) using pH meter and bring final volume to 100 ml with water.

3. *Alkaline preincubation* (18 mM

CaCl₂ in 100 mM buffer, pH 10.4)

Sigma No. 221 buffer (1.5 M)	3.35ml
CaCl ₂ (0.18M)	5ml
Distilled water	40ml

Adjust pH to 10.4 with KOH or HCl (1-10N) using pH meter and bring final volume to 50ml with water.

4. *Incubation solution* (2.7 mM ATP,

50mM KCl, 18 mM CaCl₂ in 100 mM buffer, pH 9.4)

Sigma No. 221 buffer (1.5 M)	3.35ml
CaCl ₂ (0.18 M)	5ml
KCl (MW 75)	185mg
ATP disodium (MW 551.2)	76mg
Distilled water	40ml

Adjust pH to 9.4 with 6N HCl or KOH using pH meter and bring final volume to 50 ml with water

5. *Wash solution* (1% CaCl₂)

CaCl ₂ (MW 147)	2g
Distilled water	200ml

6. *Cobalt chloride solution* (2%)

CoCl ₂ (MW 238)	1g
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Distilled water	50ml
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7. *Alkaline washing solution*

(100 mM buffer, pH 9.4)

Sigma No. 221 buffer (1.5 M)	13.4ml
------------------------------	--------

Distilled water	160ml
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Bring pH to 9.4 with HCl (1-6 N) using pH meter and adjust to final volume of 200 ml with water.

8. *Ammonium sulfide solution* (1%)

Ammonium sulfide (light)	0.5ml
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Distilled water	50ml
-----------------	------

9. *Acid preincubation* (50mM potassium

acetate, 18 mM CaCl_2 , pH 4.3 and/or 4.5)

CaCl_2 (p.18 M)	10ml
--------------------------	------

Glacial acetic acid	.3ml
---------------------	------

Distilled water	90ml
-----------------	------

Adjust pH to 4.3 or 4.5 with KOH (1-5N) using pH meter and bring final volume to 100ml with water.

Sigma Buffer solution (1.5 M)

Sigma No. 221 (MW 89.1)	13.365g
-------------------------	---------

Distilled water	100ml
-----------------	-------

Solutions 1, 2, 5 and 9 are stable and therefore large quantities can be prepared and stored in the refrigerator. The other solutions are not stable and are prepared immediately before use. The pH of buffer solutions in the alkaline range (solutions 3,4 and 7) is not stable because

the solutions tend to absorb CO_2 from the atmosphere. These solutions must therefore be prepared immediately before use.

Sigma No.221 buffer is a trade name for a 1.5 M solution of 2-amino-2-methyl-1-propanol that is obtainable from the Sigma Chemical Co., 3500 DeKalb Street, St. Louis, Missouri, 63118. Ammonium sulfide (light) deteriorates with age. It should be replaced if mottled, uneven staining of the tissue sections occurs.

APPENDIX C

TABLES OF STATISTICAL DATA - LUMBAR DISC DISORDER: AFFECTED vs NONAFFECTED

KEY:

PROPORTION OF FIBER TYPES

ASP1 - affected superficial percentage type I
NSP1 - nonaffected superficial percentage type I
ADP1 - affected deep percentage type I
NDP1 - nonaffected deep percentage type I

ASP2 - affected superficial percentage type II
NSP2 - nonaffected superficial percentage type II
ADP2 - affected deep percentage type II
NDP2 - nonaffected deep percentage type II

SIZE OF FIBER TYPES

ASS1 - affected superficial size type I
NSS1 - nonaffected superficial size type I
ADS1 - affected deep size type I
NDS1 - nonaffected deep size type I

ASS2 - affected superficial size type II
NSS2 - nonaffected superficial size type II
ADS2 - affected deep size type II
NDS2 - nonaffected deep size type II

STRENGTH FACTOR COMPONENT OF FIBER TYPES (x1/1000)

ASF1 - affected superficial strength factor type I
NSF1 - nonaffected superficial strength factor type I
ADF1 - affected deep strength factor type I
NDF1 - nonaffected deep strength factor type I

ASF2 - affected superficial strength factor type II
NSF2 - nonaffected superficial strength factor type II
ADF2 - affected deep strength factor type II
NDF2 - nonaffected deep strength factor type II

LUMBAR DISC - PROPORTION OF FIBER TYPE I & II

- affected versus nonaffected (n=18)
- significant difference (*) in proportion
of type I and II fibers on opposite sides of
the column

REGION	MEAN	S.D.	2-TAIL PROB
ASP1	58.67	20.5	not significant
NSP1	52.72	14.1	0.349
ADP1	52.94	15.0	not significant
NDP1	49.39	19.5	0.509
ASP2	41.33	20.5	not significant
NSP2	47.28	14.1	0.349
ADP2	47.06	15.0	not significant
NDP2	50.61	19.5	0.509

LUMBAR DISC - SIZE OF FIBER TYPE I & II

- affected versus nonaffected (n=18)
- significant difference (*) in size of type I and II fibers on opposite sides of the column

REGION	MEAN	S.D.	2-TAIL PROB
ASS1	56.64	11.7	not significant
NSS1	62.01	12.9	0.181
ADS1	56.91	14.9	not significant
NDS1	58.76	8.3	0.632
ASS2	37.89	11.3	not significant
NSS2	37.17	8.4	0.692
ADS2	42.51	14.2	not significant
NDS2	40.82	12.8	0.690

LUMBAR DISC - STRENGTH FACTOR FIBER TYPE I & II

- affected versus nonaffected (n=18)
- significant difference (*) in potential strength factor component of type I and II fibers on opposite sides of the column

REGION	MEAN	S.D.	2-TAIL PROB
ASF 1	148.13	58.0	not significant
NSF 1	157.49	54.8	0.592
ADF 1	141.89	80.0	not significant
NDF 1	144.17	62.5	0.928
ASF 2	51.18	40.0	not significant
NSF 2	55.49	30.2	0.649
ADF 2	79.84	71.3	not significant
NDF 2	79.94	68.8	0.996

APPENDIX D

TABLES OF STATISTICAL DATA - LUMBAR DISC DISORDER: LEFT vs RIGHT

KEY:

PROPORTION OF FIBER TYPES

LSP1 - left superficial percentage type I
RSP1 - right superficial percentage type I
LDP1 - left deep percentage type I
RDP1 - right deep percentage type I

LSP2 - left superficial percentage type II
RSP2 - right superficial percentage type II
LDP2 - left deep percentage type II
RDP2 - right deep percentage type II

SIZE OF FIBER TYPES

LSS1 - left superficial size type I
RSS1 - right superficial size type I
LDS1 - left deep size type I
RDS1 - right deep size type I

LSS2 - left superficial size type II
RSS2 - right superficial size type II
LDS2 - left deep size type II
RDS2 - right deep size type II

STRENGTH FACTOR COMPONENT OF FIBER TYPES (x1/1000)

LSF1 - left superficial strength factor type I
RSF1 - right superficial strength factor type I
LDF1 - left deep strength factor type I
RDF1 - right deep strength factor type I

LSF2 - left superficial strength factor type II
RSF2 - right superficial strength factor type II
LDF2 - left deep strength factor type II
RDF2 - right deep strength factor type II

A. LEFT vs RIGHT: COMPLETE SAMPLE

LUMBAR DISC - PROPORTION OF FIBER TYPE I & II

- left versus right (n=19)
- significant difference (*) in proportion
of type I and II fibers on opposite sides of
the column

REGION	MEAN	S.D.	2-TAIL PROB
LSP1	61.42	18.8	*
RSP1	50.05	13.5	0.049
LDP1	47.11	19.5	not significant
RDP1	54.68	14.0	0.147
LSP2	38.58	18.8	*
RSP2	49.95	13.5	0.049
LDP2	52.89	19.5	not significant
RDP2	45.3	14.0	0.147

LUMBAR DISC - SIZE OF FIBER TYPE I & II

- left versus right (n=19)
- significant difference (*) in size of type I and II fibers on opposite sides of the column

REGION	MEAN	S.D.	2-TAIL PROB
LSS1	55.07	14.3	not significant
RSS1	62.23	9.3	0.052
LDS1	56.99	7.8	not significant
RDS1	57.64	15.0	0.859
LSS2	36.26	10.7	not significant
RSS2	37.91	8.9	0.331

LUMBAR DISC - POTENTIAL STRENGTH FACTOR TYPE I & II

- left versus right (n=19)
- significant difference (*) in potential strength factor component of type I and II fibers on opposite sides of the column

REGION	MEAN	S.D.	2-TAIL PROB
LSF 1	140.80	54.1	not significant
RSF 1	158.82	67.0	0.269
LDF 1	129.28	57.1	not significant
RDF 1	150.57	81.7	0.369
LSF 2	44.70	40.8	not significant
RSF 2	59.42	25.8	0.088
LDF 2	82.24	63.2	not significant
RDF 2	82.98	75.1	0.970

B. LEFT vs RIGHT SIDE, MALES vs FEMALES

LUMBAR DISC - PROPORTION OF FIBER TYPE I & II

- left versus right
- males (n=12) versus females (n=7)
- males=group 1; females=group 2
- significant difference (*) in proportion of type I and II fibers on similar sides of the column

REGION	GROUP	MEAN	S.D.	2-TAIL PROB
LSP1	1	59.58	22.2	not significant
	2	64.57	11.6	0.529
LSP2	1	40.42	22.2	not significant
	2	35.43	11.6	0.529
LDP1	1	40.92	21.5	*
	2	57.71	9.3	0.031
LDP2	1	59.08	21.5	*
	2	42.29	9.3	0.031
RSP1	1	47.92	15.8	not significant
	2	53.71	8.2	0.308
RSP2	1	52.08	15.8	not significant
	2	46.29	8.2	0.308
RDP1	1	52.67	16.9	not significant
	2	58.14	6.7	0.334
RDP2	1	47.33	16.9	not significant
	2	41.86	16.9	0.334

LUMBAR DISC - SIZE OF FIBER TYPE I & II

- left versus right
- males (n=12) versus females (n=7)
- males=group 1; females=group 2
- significant difference (*) in size of type I and II fibers on similar sides of the column

REGION	GROUP	MEAN	S.D.	2-TAIL PROB
LSS1	1	57.79	17.0	not significant
	2	50.40	6.4	0.197
LSS2	1	41.12	9.1	*
	2	27.93	8.1	0.008
LDS1	1	56.83	8.7	not significant
	2	57.24	6.8	0.911
LDS2	1	47.56	7.2	*
	2	30.23	7.4	0.000
RSS1	1	63.26	10.5	not significant
	2	60.47	7.3	0.506
RSS2	1	40.88	6.6	not significant
	2	32.83	10.5	0.101
RDS1	1	59.42	16.5	not significant
	2	59.64	18.2	0.979
RDS2	1	47.51	12.6	not significant
	2	37.13	18.9	0.228

LUMBAR DISC - STRENGTH FACTOR FIBER TYPE I & II

- left versus right
- males (n=12) versus females (n=7)
- males=group 1; females=group 2
- significant difference (*) in strength factor component of type I and II fibers on similar sides of the column

REGION	GROUP	MEAN	S.D.	2-TAIL PROB
LSF1	1	148.22	63.9	not significant
	2	128.13	31.3	0.372
LSF2	1	57.72	46.4	*
	2	22.39	11.4	0.026
LDF1	1	113.60	51.9	not significant
	2	153.91	60.0	0.169
LDF2	1	111.79	82.0	*
	2	31.58	13.3	0.001
RSF1	1	159.87	78.3	not significant
	2	157.00	47.4	0.922
RSF2	1	67.86	23.5	not significant
	2	44.96	24.5	0.069
RDF1	1	144.71	72.5	not significant
	2	169.18	97.1	0.575
RDF2	1	98.50	79.5	not significant
	2	56.37	63.7	0.224

APPENDIX E

TABLES OF STATISTICAL DATA - ADOLESCENT IDIOPATHIC SCOLIOSIS

PROPORTION OF FIBER TYPES

KEY:

ACSP - above concave superficial percentage type I
 AXSP - above convex superficial percentage type I
 ACDP - above concave deep percentage type I
 AXDP - above convex deep percentage type I
 XCSP - apex concave superficial percentage type I
 XXSP - apex convex superficial percentage type I
 XCDP - apex concave deep percentage type I
 XXDP - apex convex deep percentage type I
 BCSP - below concave superficial percentage type I
 BXSP - below convex superficial percentage type I
 BCDP - below concave deep percentage type I
 BXDP - below convex deep percentage type I

Significant difference (*) in proportion
 of type I fibers on opposite sides of the column.

REGION	MEAN	S.D.	2-TAIL PROB
ACSP	57.43	10.1	*
AXSP	74.00	8.76	0.009
ACDP	59.29	13.9	not significant
AXDP	64.29	10.0	0.413
XCSP	60.86	16.4	not significant
XXSP	66.57	6.1	0.413
XCDP	55.57	16.6	*
XXDP	69.57	11.36	0.017
BCSP	57.43	15.1	not significant
BXSP	66.57	15.5	0.161
BCDP	55.00	12.6	not significant
BXDP	60.00	12.3	0.275

SCOLIOSIS - SIZE OF FIBER TYPE I

KEY:

ACS1 - above concave superficial type I
 AXS1 - above convex superficial type I
 ACD1 - above concave deep type I
 AXD1 - above convex deep type I

XCS1 - apex concave superficial type I
 XXS1 - apex convex superficial type I
 XCD1 - apex concave deep type I
 XXD1 - apex convex deep type I

BCS1 - below concave superficial type I
 BXS1 - below convex superficial type I
 BCD1 - below concave deep type I
 BXD1 - below convex deep type I

Significant difference (*) in size of
 type I fibers on opposite sides of the column

REGION	MEAN	S.D.	2-TAIL PROB
ACS1	43.39	7.6	not significant
AXS1	48.86	9.9	0.152
ACD1	46.44	8.6	not significant
AXD1	49.23	5.4	0.553
XCS1	45.67	7.5	not significant
XXS1	50.83	3.9	0.103
XCD1	44.87	5.9	*
XXD1	52.76	9.2	0.011
BCS1	41.19	6.0	*
BXS1	54.81	9.5	0.008
BCD1	50.27	11.6	not significant
BXD1	58.59	17.4	0.117

SCOLIOSIS - SIZE OF FIBER TYPE II

KEY:

ACS2 - above concave superficial type II

AXS2 - above convex superficial type II

ACD2 - above concave deep type II

AXD2 - above convex deep type II

XCS2 - apex concave superficial type II

XXS2 - apex convex superficial type II

XCD2 - apex concave deep type II

XXD2 - apex convex deep type II

BCS2 - below concave superficial type II

BXS2 - below convex superficial type II

BCD2 - below concave deep type II

BXD2 - below convex deep type II

Significant difference (*) in size of
type II fibers on opposite sides of the column

REGION	MEAN	S.D.	2-TAIL PROB
ACS2	40.59	6.8	not significant
AXS2	41.09	12.1	0.931
ACD2	44.33	9.8	not significant
AXD2	40.64	6.1	0.371
XCS2	38.89	10.0	not significant
XXS2	41.01	5.6	0.680
XCD2	41.83	11.3	not significant
XXD2	43.1	10.1	0.763
BCS2	35.6	8.1	not significant
BXS2	32.76	8.2	0.203
BCD2	39.29	8.5	not significant
BXD2	41.83	10.5	0.441

SCOLIOSIS - POTENTIAL STRENGTH FACTOR TYPE I

KEY:

ACSF1 - above concave superficial S.F. type I
 AXSF1 - above convex superficial S.F. type I
 ACDF1 - above concave deep S.F. type I
 AXDF1 - above convex deep S.F. type I

XCSF1 - apex concave superficial S.F. type I
 XXSF1 - apex convex superficial S.F. type I
 XCDF1 - apex concave deep S.F. type I
 XXDF1 - apex convex deep S.F. type I

BCSF1 - below concave superficial S.F. type I
 BXSF1 - below convex superficial S.F. type I
 BCDF1 - below concave deep S.F. type I
 BXDF1 - below convex deep S.F. type I

Significant difference (*) in potential
 strength factor component of type I fibers on
 opposite sides of the column

REGION	MEAN x1/1000	S.D. x1/1000	2-TAIL PROB
ACSF1	88.21	36.6	*
AXSF1	143.53	53.3	0.041
ACDF1	107.60	59.1	not significant
AXDF1	123.46	31.9	0.601
XCSF1	96.71	27.7	*
XXSF1	135.59	23.4	0.011
XCDF1	85.70	24.0	*
XXDF1	150.53	37.9	0.003
BCSF1	80.81	41.1	*
BXSF1	164.09	74.0	0.017
BCDF1	114.49	60.0	not significant
BXDF1	181.92	131.1	0.091

SCOLIOSIS - POTENTIAL STRENGTH FACTOR TYPE II

KEY:

ACSF2 - above concave superficial S.F. type II
 AXSF2 - above convex superficial S.F. type II
 ACDF2 - above concave deep S.F. type II
 AXDF2 - above convex deep S.F. type II

XCSF2 - apex concave superficial S.F. type II
 XXSF2 - apex convex superficial S.F. type II
 XCDF2 - apex concave deep S.F. type II
 XXDF2 - apex convex deep S.F. type II

BCSF2 - below concave superficial S.F. type II
 BXSF2 - below convex superficial S.F. type II
 BCDF2 - below concave deep S.F. type II
 BXDF2 - below convex deep S.F. type II

Significant difference (*) in potential
 strength factor component of type II fibers
 on opposite sides of the column

REGION	MEAN x1/1000	S.D. x1/1000	2-TAIL PROB
ACSF2	56.75	27.9	not significant
AXSF2	35.78	24.9	0.148
ACDF2	64.03	32.5	not significant
AXDF2	47.52	19.0	0.125
XCSF2	48.27	29.6	not significant
XXSF2	45.84	17.1	0.871
XCDF2	65.6	44.8	not significant
XXDF2	50.1	36.8	0.302
BCSF2	40.81	18.6	not significant
BXSF2	29.71	21.2	0.137
BCDF2	55.73	27.5	not significant
BXDF2	53.45	20.9	0.868

APPENDIX F

GROSS MORPHOLOGY OF MONKEY PARAVERTEBRAL MUSCLES

Review of the literature concerning monkey paravertebral musculature revealed the existence of major gaps in the available information. Investigators have tended to be relatively general in their descriptions and illustrations and have used different animal species or nomenclature. In the following table general categories into which the back muscles have been divided by the authors are included.

These categories are: a. Spino-occipital system; b. Long system; c. Spino-spinal system; d. Transversospinal system; e. Intervertebral system; f. Levatores costarum system. Excluded from these categories in the following table are the extensor and abductor muscles of the tail of the monkey.

The Long system, frequently termed *Erector or Extensor Spinae* is divisible into a lateral iliocostalis muscle and a more medial longissimus muscle as in the human. These two muscles are barely divisible in the lumbar region and are intimately connected with the lumbodorsal aponeurosis (LDA) covering spinalis, longissimus and part of iliocostalis lumborum. This aponeurosis arises from the spines of the sacral and lumbar vertebrae.

INTRINSIC (DEEP) BACK MUSCULATURE OF THE RHESUS MONKEY*

C,T,L,S = cervical, thoracic, lumbar, sacral vertebrae

TP = transverse process(es); SP = spinous process(es)

ArP = articular process(es); AcP = accessory process(es)

MaP = mamillary process(es); ESA = erector spinae

aponeurosis; LDA = lumbo dorsal aponeurosis

* Taken from Hartman and Straus, 1933.

MUSCLE ATTACHMENTS

a. Spino-occipital system

SPLENIUS	From midline between the inion and the fourth thoracic spine to superior nuchal line and the mastoid process
----------	--

b. Long system

ILIOCOSTALIS

-lumborum	From the crest of the ilium and lateral border of longissimus to lumbar TP and ribs 8-12
-thoracis	As muscle bundles (7 or 8) from all the lower ribs to the upper 6-7 ribs and C7TP (this latter portion having secondary attachment to the first 4 ribs as well)

LONGISSIMUS

-thoracis	From the medial part of the iliac tubercle and deep surface of the LDA to the lumbar AcP; thoracic AcP and adjacent ribs; and C2-7TP.
-cervicis	From T1-5TP to C2-7TP
-capitis	From T1-5TP and C3(4)-7TP to mastoid

process just deep to lateral border
of Splenius

c. Spinothal system

SPINALIS

- lumborum From the deep surface of the LDA
to lumbar SP
- thoracis From the superior surface of the LDA
in the lower thoracic region (8-11
ribs) to C7, T1-6SP (or more)
- cervicis May be present in the form of a few
fibers extending between C2-T1SP

d. Transversospinal system

SEMISPINALIS From TP to SP spanning more than
4 vertebrae. Present only in the upper
thoracic and cervical regions.

-cervicis From cervical TP to C2-7SP

-capitis Medial: BIVENTER CERVICIS - From
T3(4)-6(7)TP to medial quarter or
third of the superior nuchal line

Lateral: COMPLEXUS - From C2(3)-7TP and
T1-2(3)TP to medial half or two-thirds
of the superior nuchal line

MULTIFIDUS Bridge 2-4 vertebral spaces and extend
entire length of the vertebral column.
From TP to SP and are inseparably fused
for the most part with Semispinalis

ROTATOIRES From TP to SP. More obvious "cranialward"

-longi Bridge 1 vertebra

-brevis Attach to adjoining SP

e. Intervertebral system

INTER

TRANSVERSARI I From TP to TP and mostly tendinous. Prominent as fleshy bundles in the cervical region and particularly in the upper cervical region where they may extend over 2-3 vertebral spaces. In the lumbar region they consist of two parts; those between the mamillary processes and those passing more ventrally from mamillary processes to the preceding accessory processes as far forward as approximately T9 vertebra.

INTERSPINALES Connect adjoining SP and are for the most part ligamentous demonstrating few muscle fibers.

NB: Excluded from this category are the sub-occipital muscles

f. Levatores costarum system

LEVATORES

COSTARUM C7-T11 TP to the rostral border of one rib below. The longer element spanning two costal spaces, such as occurs in the human, is absent.

* Taken from Hartman and Straus, 1933.

APPENDIX G

TABLES OF STATISTICAL DATA - RHESUS MONKEY: LEFT vs RIGHT PROPORTION OF FIBER TYPES

KEY:

T3 ALSP - above left superficial percentage type I
 ARSP - above right superficial percentage type I
 ALDP - above left deep percentage type I
 ARDP - above right deep percentage type I

T8 XLSP - apex left superficial percentage type I
 XRSP - apex right superficial percentage type I
 XLDP - apex left deep percentage type I
 XRDP - apex right deep percentage type I

L3 BLSP - below left superficial percentage type I
 BRSP - below right superficial percentage type I
 BLDP - below left deep percentage type I
 BRDP - below right deep percentage type I

Significant difference (*) in proportion
 of type I fibers on opposite sides of the column.

REGION	MEAN	S.D.	2-TAIL PROB
ALSP	35.20	8.7	not significant
ARSP	38.00	6.5	0.377
ALDP	50.00	6.6	not significant
ARDP	53.20	7.8	0.078
XLSP	28.00	4.4	not significant
XRSP	25.60	5.8	0.178
XLDP	42.60	9.3	not significant
XRDP	51.00	9.7	0.104
BLSP	20.80	3.6	not significant
BRSP	24.40	3.3	0.266
BLDP	46.40	10.4	not significant
BRDP	40.80	6.1	0.268

MONKEY - SIZE OF FIBER TYPE I

KEY:

T3 ALS1 - above left superficial type I
 ARS1 - above right superficial type I
 ALD1 - above left deep type I
 ARD1 - above right deep type I

T8 XLS1 - apex left superficial type I
 XRS1 - apex right superficial type I
 XLD1 - apex left deep type I
 XRD1 - apex right deep type I

L3 BLS1 - below left superficial type I
 BRS1 - below right superficial type I
 BLD1 - below left deep type I
 BRD1 - below right deep type I

Significant difference (*) in size of
 type I fibers on opposite sides of the column

REGION	MEAN	S.D.	2-TAIL PROB
ALS1	44.36	3.5	not significant
ARS1	43.20	7.1	0.644
ALD1	44.72	4.1	not significant
ARD1	41.22	8.3	0.412
XLS1	41.76	3.3	not significant
XRS1	41.48	6.3	0.923
XLD1	42.02	6.5	not significant
XRD1	46.00	5.3	0.411
BLS1	41.30	5.9	not significant
BRS1	40.40	7.2	0.864
BLD1	43.04	2.4	not significant
BRD1	38.34	7.1	0.214

MONKEY - SIZE OF FIBER TYPE II

KEY:

T3 ALS2 - above left superficial type II
 ARS2 - above right superficial type II
 ALD2 - above left deep type II
 ARD2 - above right deep type II

T8 XLS2 - apex left superficial type II
 XRS2 - apex right superficial type II
 XLD2 - apex left deep type II
 XRD2 - apex right deep type II

L3 BLS2 - below left superficial type II
 BRS2 - below right superficial type II
 BLD2 - below left deep type II
 BRD2 - below right deep type II

Significant difference (*) in size of
 type II fibers on opposite sides of the column

REGION	MEAN	S.D.	2-TAIL PROB
ALS2	51.40	3.0	not significant
ARS2	51.78	7.6	0.911
ALD2	50.00	7.5	not significant
ARD2	42.62	3.5	0.120
XLS2	49.54	5.4	not significant
XRS2	49.54	3.8	1.000
XLD2	43.50	7.8	not significant
XRD2	48.26	7.1	0.308
BLS2	49.12	3.2	not significant
BRS2	46.14	6.1	0.598
BLD2	38.88	4.4	*
BRD2	47.80	2.7	0.002

MONKEY - POTENTIAL STRENGTH FACTOR TYPE I

KEY:

T3 ALSF1 - above left superficial S.F. type I
 ARSF1 - above right superficial S.F. type I
 ALDF1 - above left deep S.F. type I
 ARDF1 - above right deep S.F. type I

T8 XLSF1 - apex left superficial S.F. type I
 XRSF1 - apex right superficial S.F. type I
 XLDF1 - apex left deep S.F. type I
 XRDF1 - apex right deep S.F. type I

L3 BLSF1 - below left superficial S.F. type I
 BRSF1 - below right superficial S.F. type I
 BLDF1 - below left deep S.F. type I
 BRDF1 - below right deep S.F. type I

Significant difference (*) in **potential strength factor component** of type I fibers on opposite sides of the column

REGION	MEAN x1/1000	S.D. x1/1000	2-TAIL PROB
ALSF1	54.36	13.9	not significant
ARSF1	56.60	17.9	0.774
ALDF1	79.05	16.9	not significant
ARDF1	74.02	30.9	0.732
XLSF1	38.65	9.06	not significant
XRSF1	33.94	8.08	0.252
XLDF1	61.08	25.2	not significant
XRDF1	83.48	13.1	0.080
BLSF1	28.79	11.3	not significant
BRSF1	31.65	10.5	0.765
BLDF1	67.53	16.6	not significant
BRDF1	36.76	17.8	0.022

MONKEY - POTENTIAL STRENGTH FACTOR TYPE II

KEY:

T3 ALSF2 - above left superficial S.F. type II
 ARSF2 - above right superficial S.F. type II
 ALDF2 - above left deep S.F. type II
 ARDF2 - above right deep S.F. type II

T8 XLSF2 - apex left superficial S.F. type II
 XRSF2 - apex right superficial S.F. type II
 XLDF2 - apex left deep S.F. type II
 XRDF2 - apex right deep S.F. type II

L3 BLSF2 - below left superficial S.F. type II
 BRSF2 - below right superficial S.F. type II
 BLDF2 - below left deep S.F. type II
 BRDF2 - below right deep S.F. type II

Significant difference (*) in potential strength factor component of type II fibers on opposite sides of the column

REGION	MEAN x1/1000	S.D. x1/1000	2-TAIL PROB
ALSF2	133.40	12.1	not significant
ARSF2	130.70	30.2	0.845
ALDF2	101.92	43.2	not significant
ARDF2	66.10	30.5	0.532
XLSF2	138.82	23.1	not significant
XRSF2	144.83	30.7	0.706
XLDF2	86.44	30.5	not significant
XRDF2	93.13	43.8	0.783
BLSF2	150.46	21.2	not significant
BRSF2	130.09	50.6	0.523
BLDF2	62.59	10.2	*
BRDF2	106.68	17.8	0.006

APPENDIX H
PHOTOGRAPHIC PLATES

THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION
PUBLISHED WEEKLY

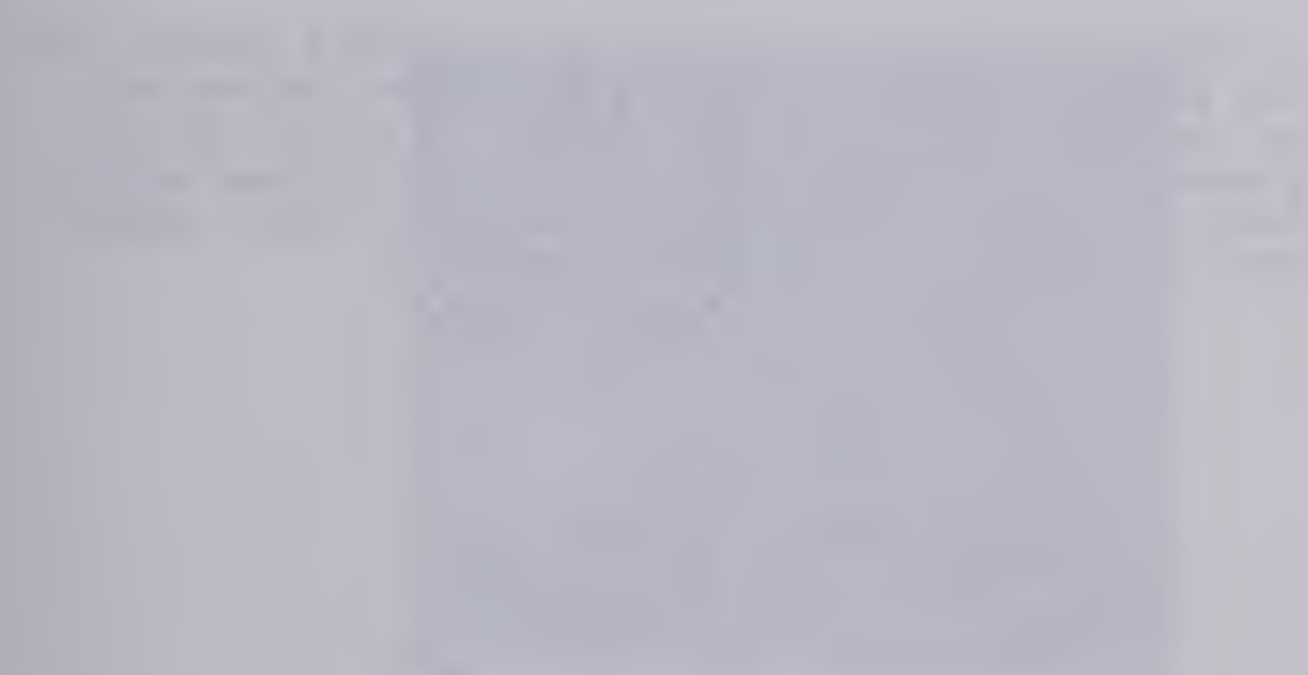


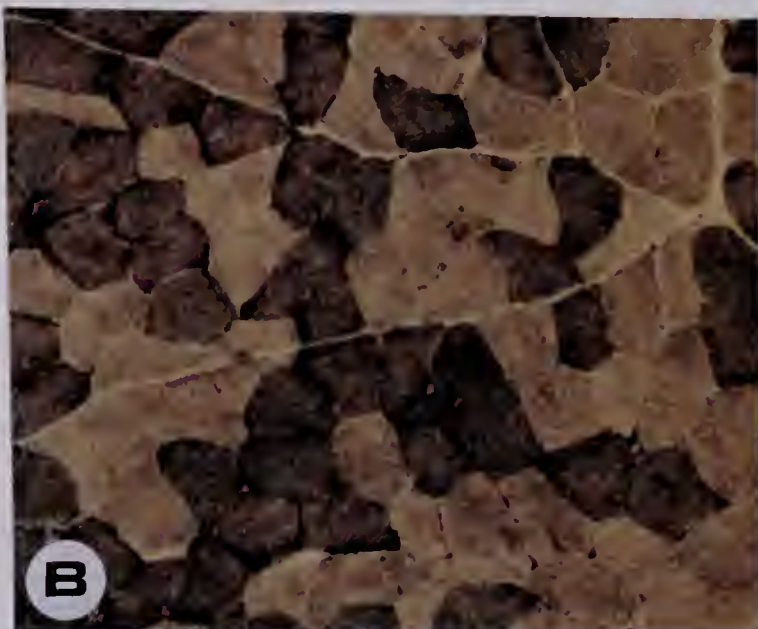
Plate 1: REACTIONS TO STAIN PROCEDURES x120

These plates illustrate relatively adjacent serial sections cut at 12 μ m from lumbar disc muscle and stained with the following procedures:

A. Masson's Trichrome. Selective demonstration of muscle fibers (red), collagen (green) and nuclei (blue-black).

B. NADH-TR. Varying degrees of blue color is deposited at the site of the enzyme activity. Myofibrils are unstained but the intermyofibrillar network is well demonstrated, type I fibers being more intensely reactive.

C. ATPase pH 10.4. A series of reactions result in the production of an end product being deposited at the site of the ATPase enzyme activity, giving a mosaic or checker-board appearance to the muscle section. Used to demonstrate specific fiber types: type I light; type II - dark.



1. The first part of the paper is devoted to the study of the properties of the function $f(x)$ defined by the equation

$$f(x) = \int_0^x \frac{1}{1+t^2} dt, \quad (1)$$

$$f(x) = \int_0^x \frac{1}{1+t^2} dt, \quad (2)$$

$$f(x) = \int_0^x \frac{1}{1+t^2} dt, \quad (3)$$

Plate 2: REACTIONS TO STAIN PROCEDURES x120

These plates illustrate relatively adjacent serial sections cut at 12 μ m from idiopathic scoliosis muscle and stained with the following procedures:

A. Masson's Trichrome.

B. NADH-TR.

C. ATPase pH 10.4.

See Plate 1 for interpretation of reactions.

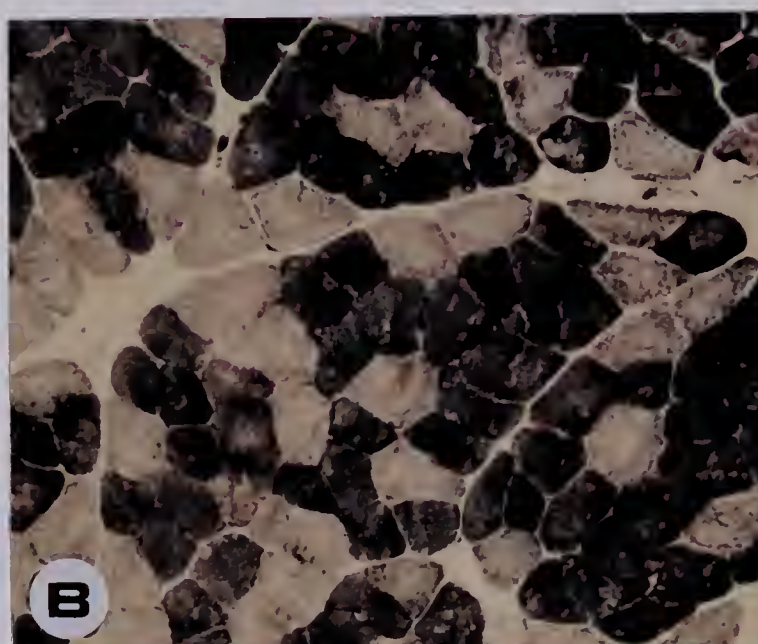
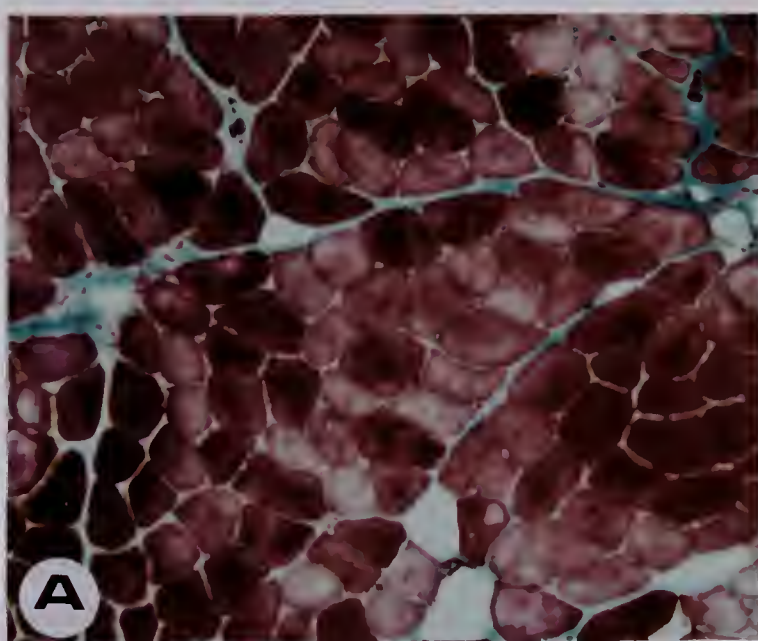


Plate 3: REACTIONS TO STAIN PROCEDURES x120

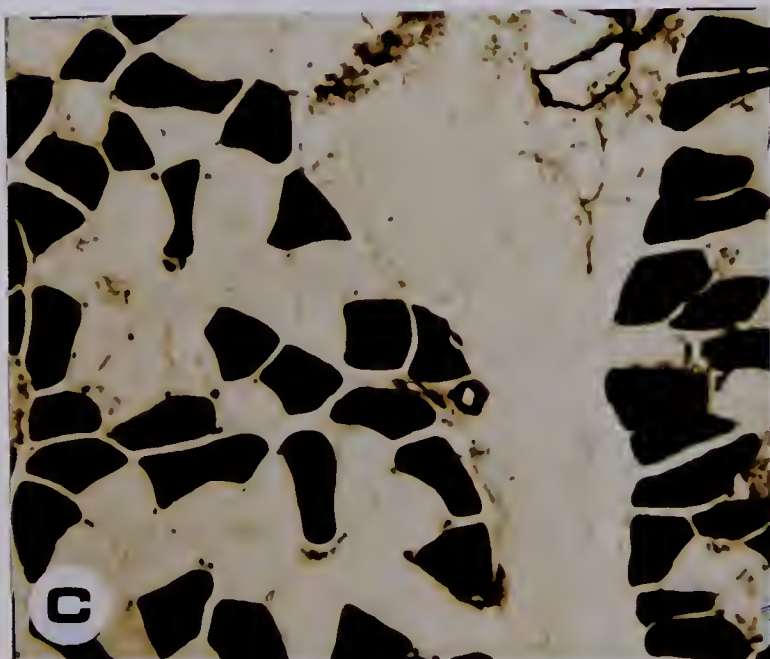
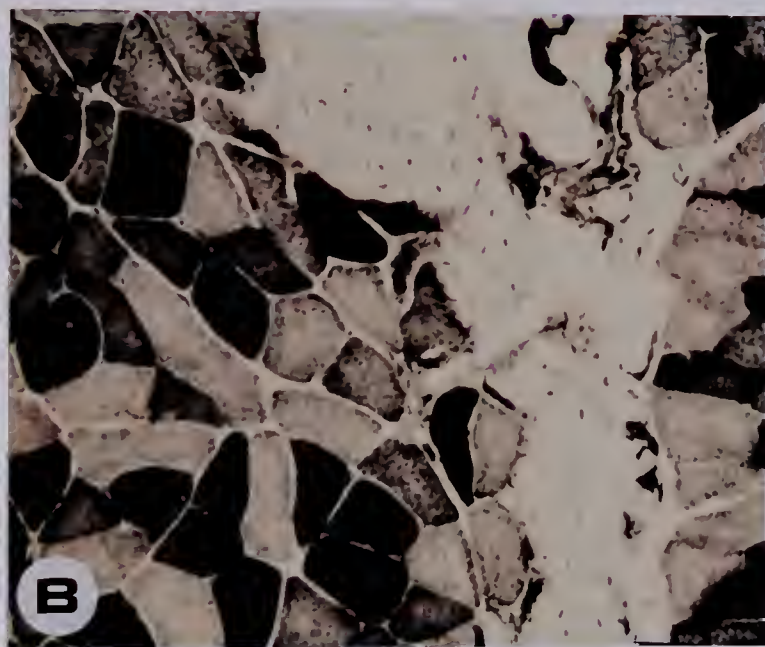
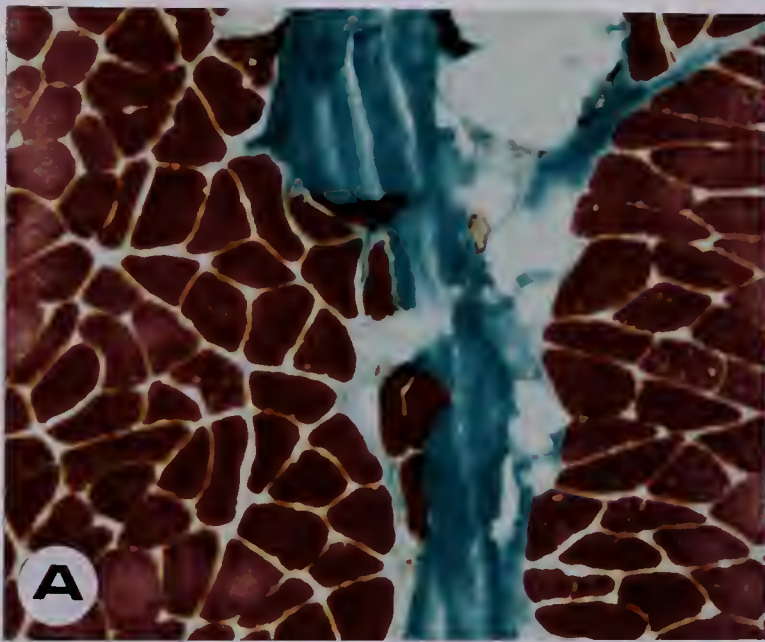
These plates illustrate relatively adjacent serial sections cut at 12 μ m from rhesus monkey muscle and stained with the following procedures:

A. Masson's Trichrome.

B. NADH-TR.

C. ATPase pH 10.4.

See Plate 1 for interpretation of reactions.



of the same kind as the
 one which I have just
 described.



See also the
 plan of the
 same building
 in the
 next page.

The plan of the building
 is shown in the next
 page. It is a very
 large and complex
 structure, and the
 plan is very
 detailed. It shows
 the various rooms
 and corridors, and
 the way in which
 they are connected
 together. The plan
 is very useful for
 understanding the
 layout of the building
 and for finding
 one's way around
 it.

Plate 4: LUMBAR DISC DISORDER - ATPase pH 10.4, x125.

A and B. Left and Right superficial group of paravertebral musculature.

Significant differences were found between type I and type II fiber type proportions on opposite sides of the column only in the superficial group. In addition type I fibers were significantly larger than type II fibers but not between equivalent measurements on the two sides of the column. See Table 3-3.

C and D. Left and Right deep group of paravertebral musculature.

No significant differences were found between equivalent measurements on the two sides of the column although type I fibers were significantly larger than type II fibers on the same side. See Table 3-3.

Type I - light

Type II - dark

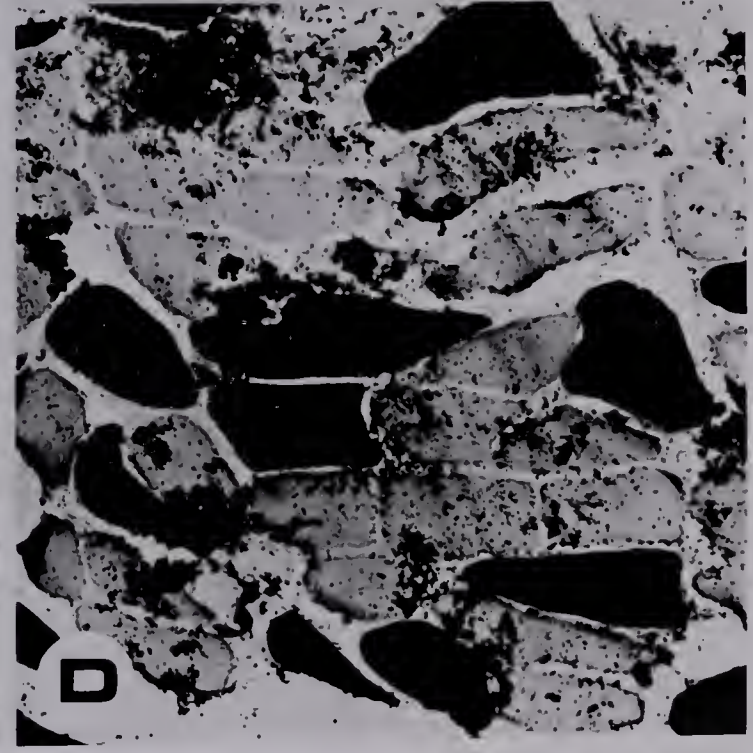
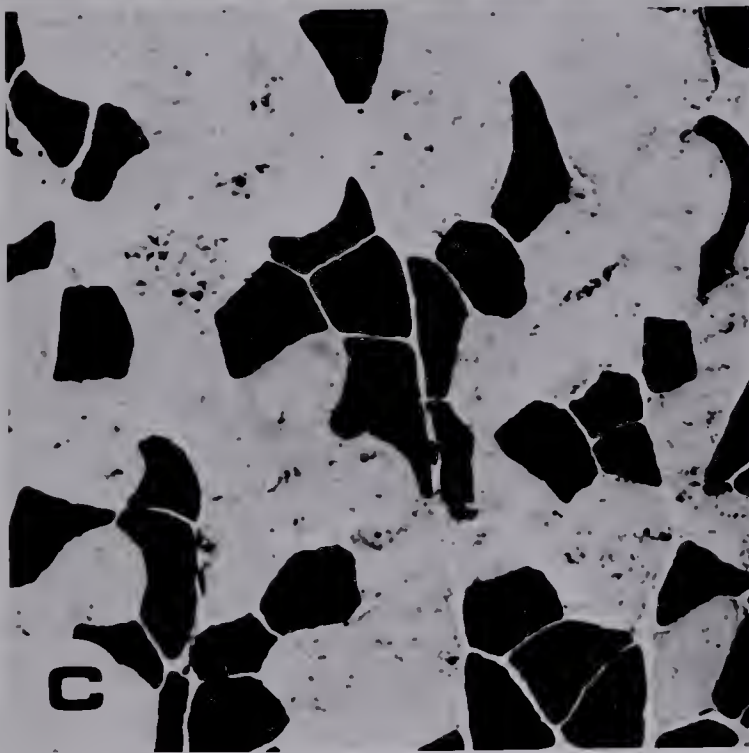
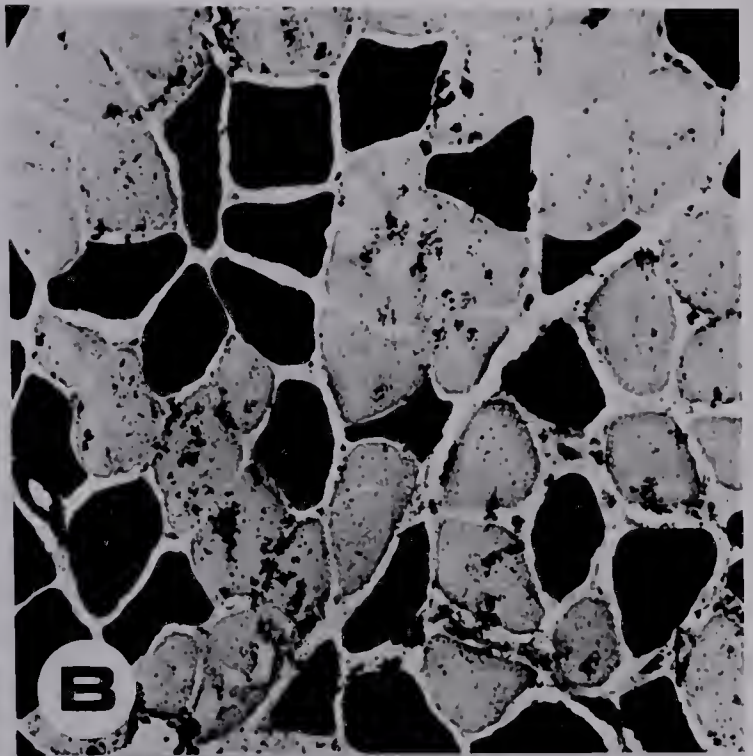


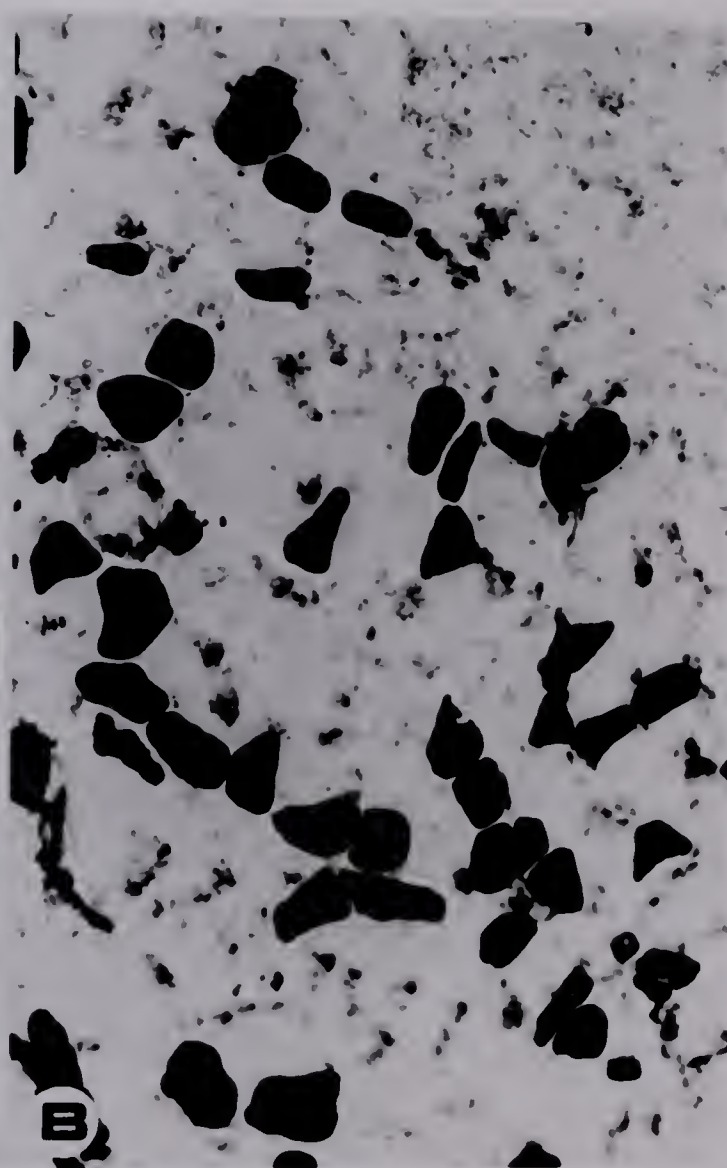
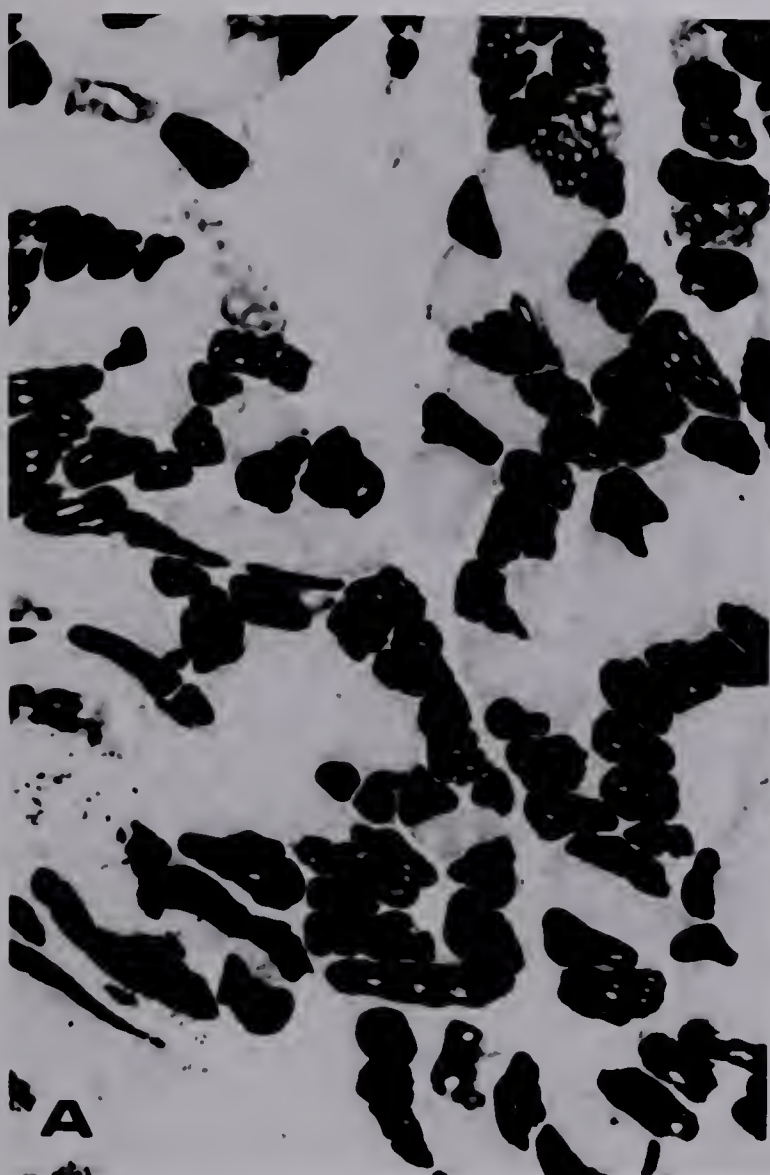
Plate 5: ADOLESCENT IDIOPATHIC SCOLIOSIS - RIGHT THORACIC
CURVATURE - ATPase pH 10.4, x125.

A and B. Concave and convex deep group of
paravertebral musculature at the level of thoracic
vertebra 11.

Significant differences were found between type I
and type II fiber type proportions at the the apex
of the major curvature on opposite sides of the
vertebral column. It is apparent in these sections
that there are more type II fibers on the concave
(left) side of the curve. See Table 3-6.

Type I - light

Type II - dark



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Plate 6: DIAGRAMMATIC SUMMARY OF MEAN SIGNIFICANT DIFFERENCES FOUND IN PARAVERTEBRAL MUSCLE BETWEEN BOTH SIDES AND IN DIFFERENT REGIONS OF THE VERTEBRAL COLUMN IN PATIENTS WITH ADOLESCENT IDIOPATHIC SCOLIOSIS

The curved lines are representative of the most common type of thoracic curvature in idiopathic scoliosis with the convexity being deviated to the right in most cases.

The dots are representative of significant differences found between equivalent superficial (S) and deep (D) muscle groups at the apex and two vertebral levels above and below the apex, on either side of the major curve. The larger dots represent a larger mean "value" and where no dots are situated, no significant differences existed between opposite sides. See Table 3-6.

A. Mean "values" for proportion of type I fibers.

B. Mean "values" for size of type I fibers.

C. Mean "values" for strength factor of type I fibers.

It will be observed that the larger values for muscle fiber characteristics are situated on the side of the convexity of the major curvature.

% TYPE 1

S D D S

A

SIZE TYPE 1

S D D S

B

SF TYPE 1

S D D S

C



Plate 7: RHESUS MONKEY SECTIONS - ATPase pH 10.4, x125.

A and B. Left and Right superficial group at Thoracic 3 vertebral level.

C and D. Left and Right superficial group at Thoracic 8 vertebral level.

E and F. Left and Right superficial group at Lumbar 3 vertebral level.

No significant differences were found in muscle fiber characteristics between sides at any vertebral level. It will be visualized that with descent of the column there is a difference to be observed in muscle fiber characteristics at different levels of the vertebral column. See Table 3-8.

Type I - light

Type II - dark; Type IIA - intermediate

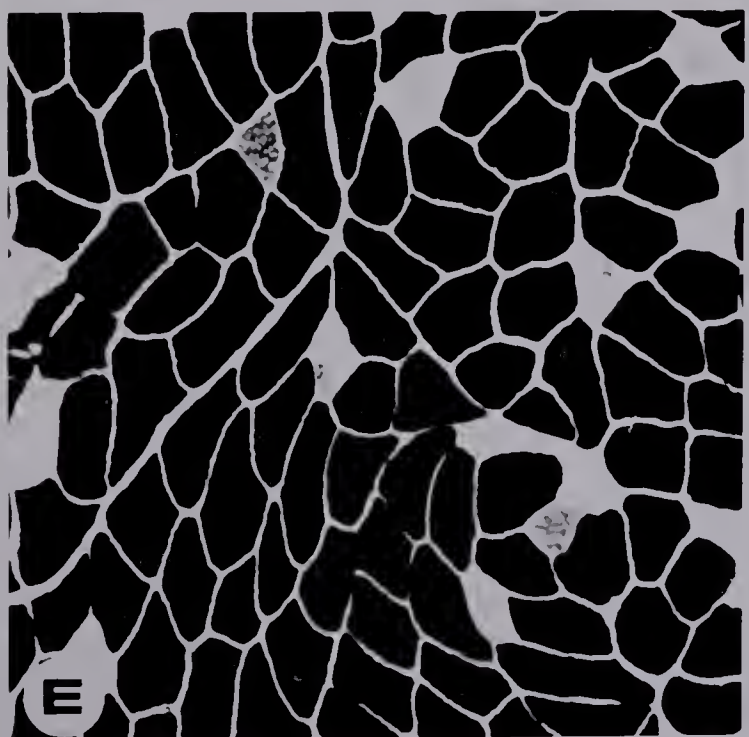
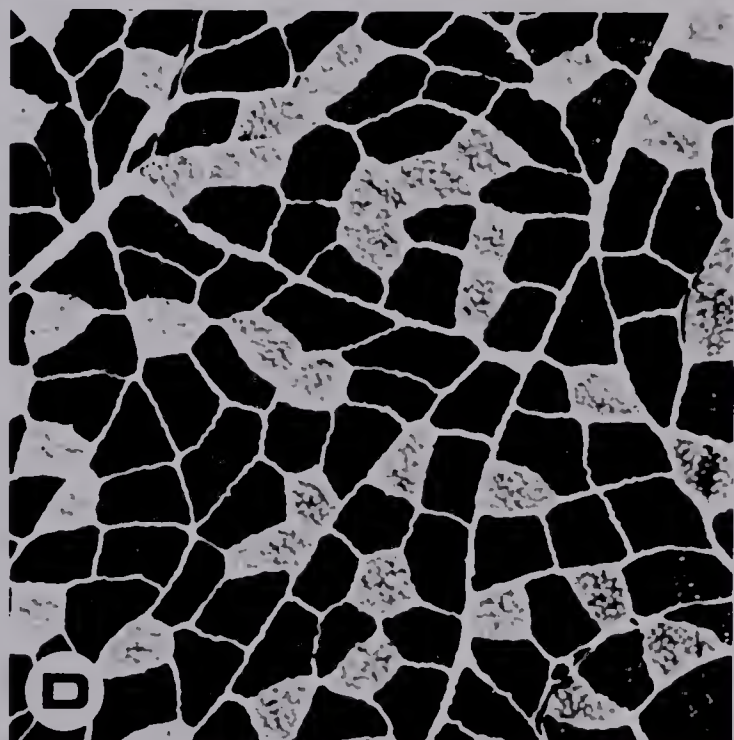
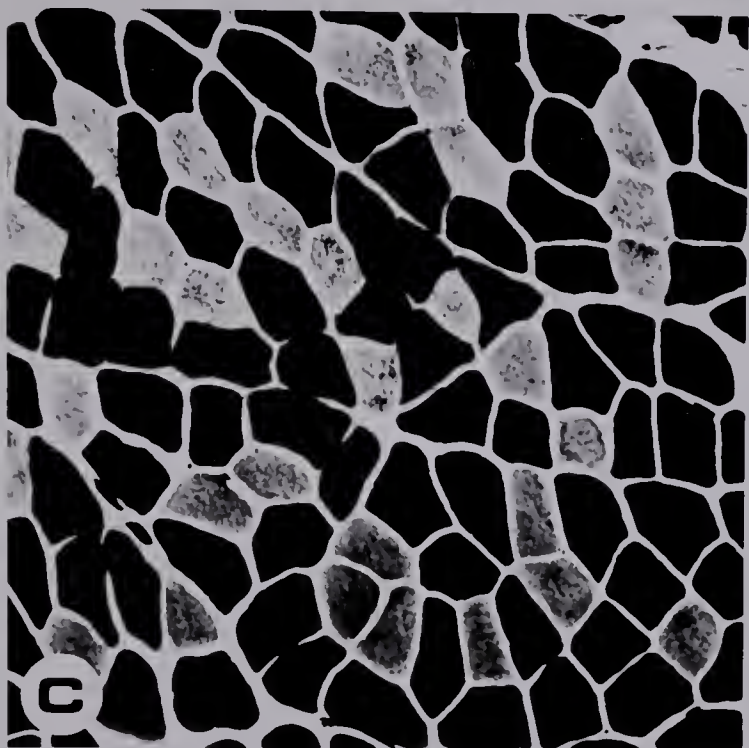
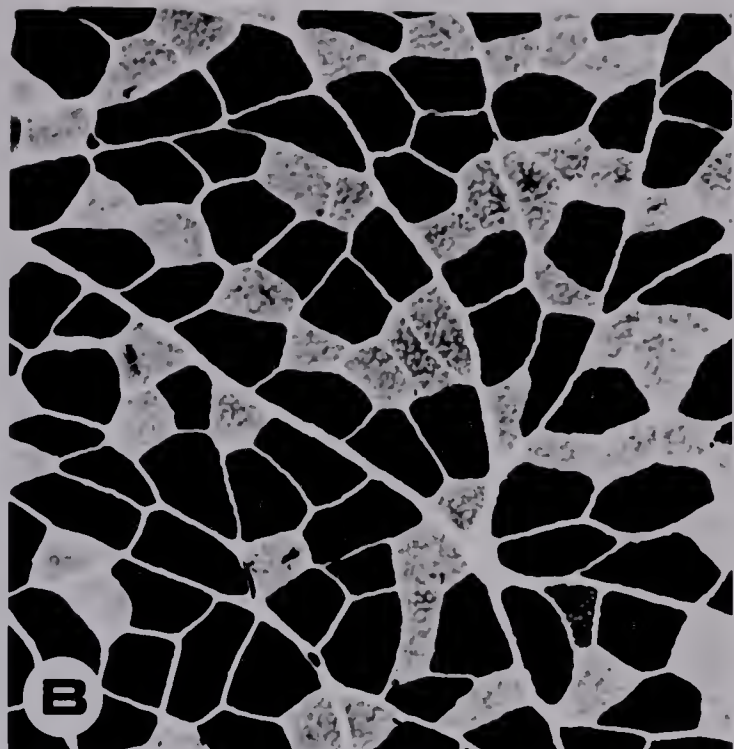
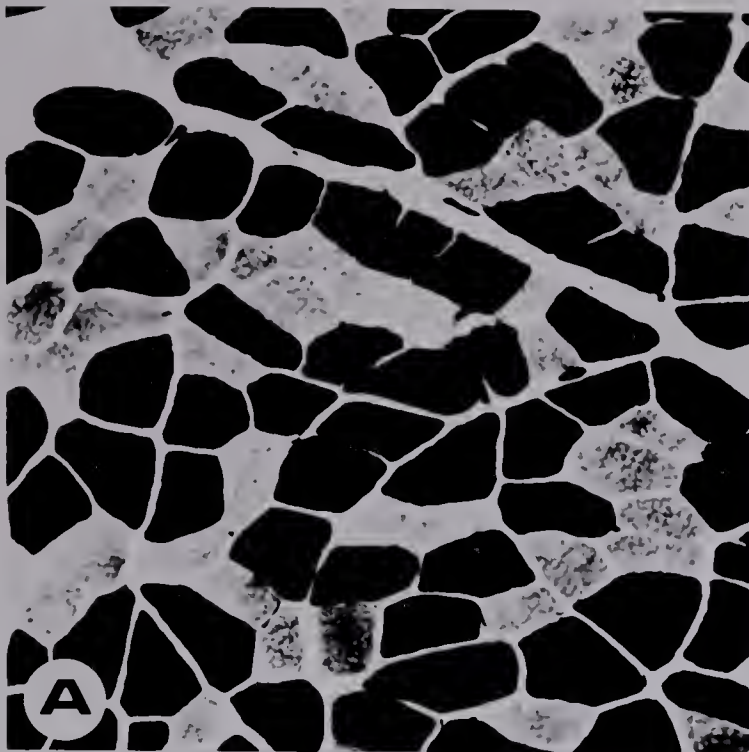




Plate 8: RHESUS MONKEY SECTIONS - ATPase pH 10.4, x125.

A and B. Left and Right deep group at Thoracic 3 vertebral level.

C and D. Left and Right deep group at Thoracic 8 vertebral level.

E and F. Left and Right deep group at Lumbar 3 vertebral level.

No significant differences were found in muscle fiber characteristics between sides of the vertebral column except in size and strength factor of type II fibers at Lumbar 3 vertebral level only. A significant decrease in percentage of type I fibers existed with descent of the vertebral column. See Table 3-8.

Type I - light

Type II - dark

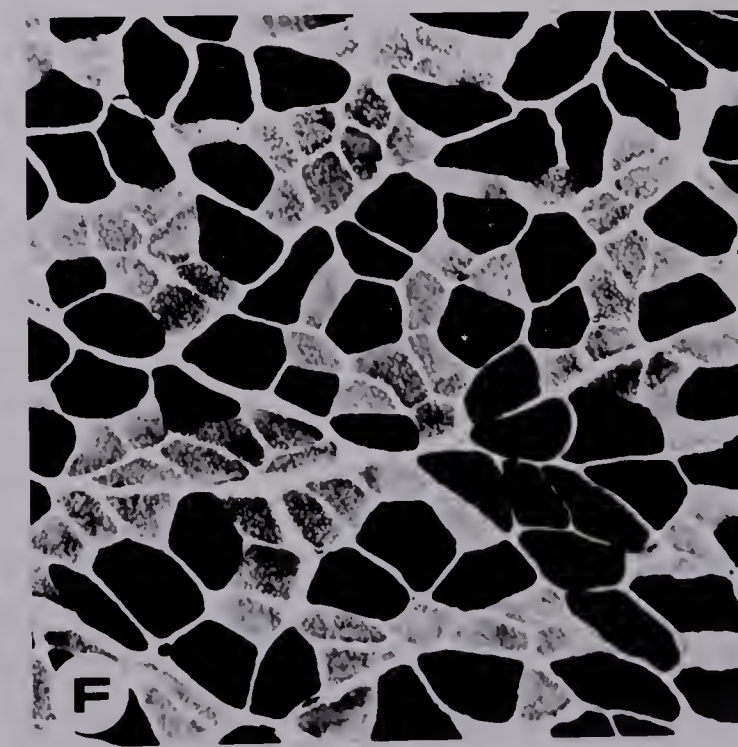
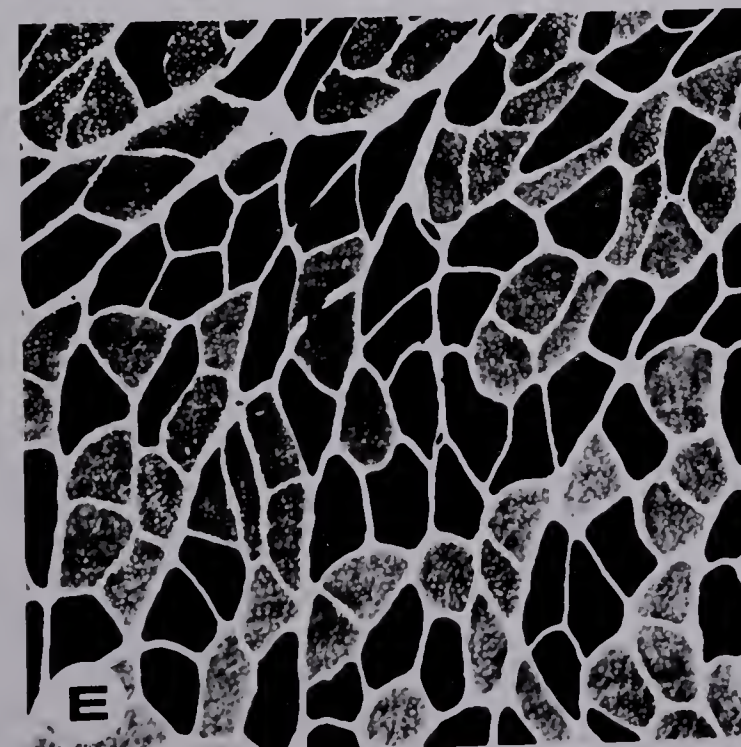
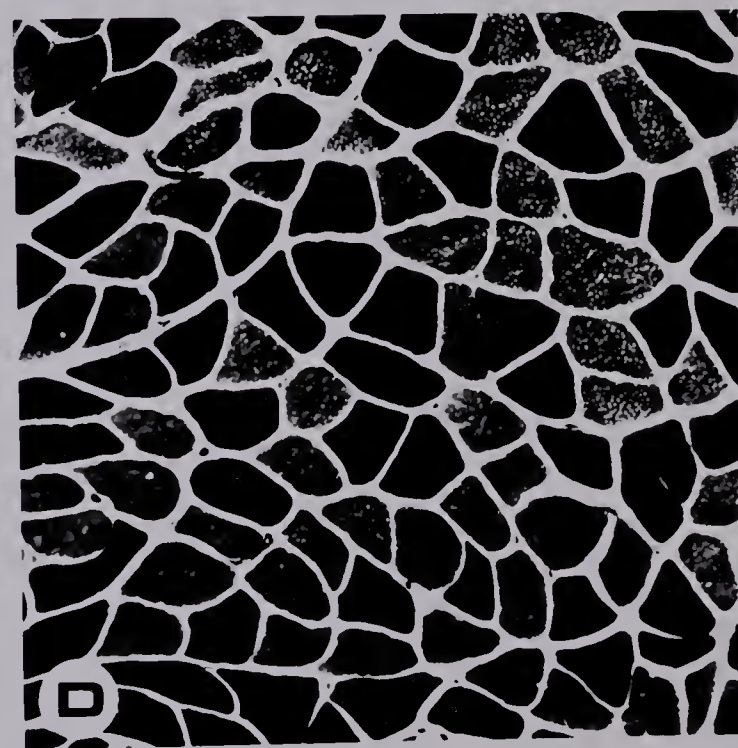
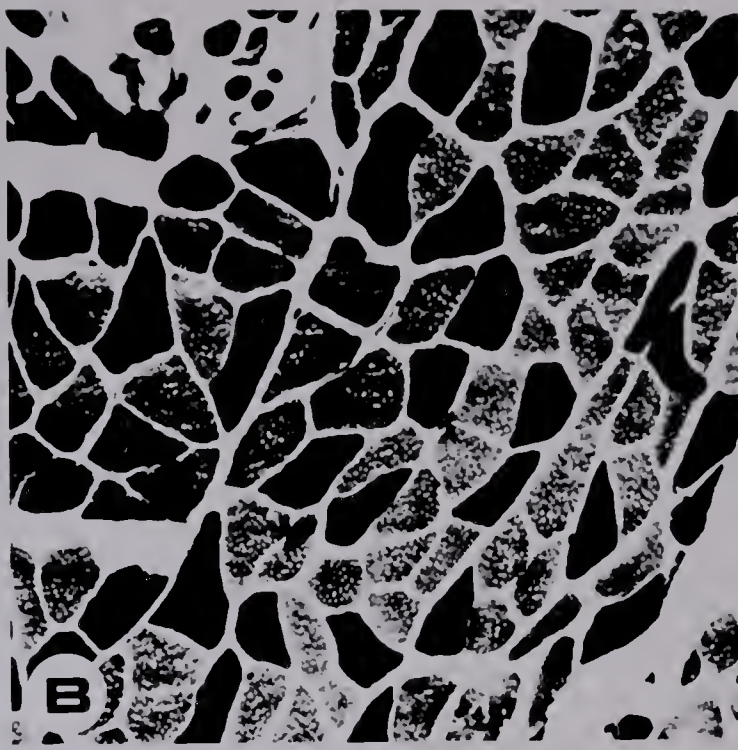
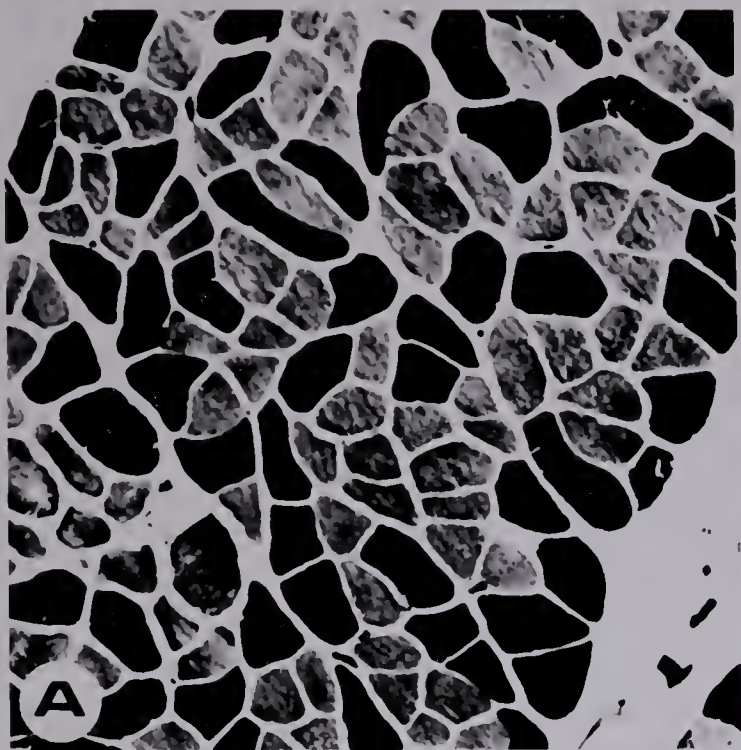
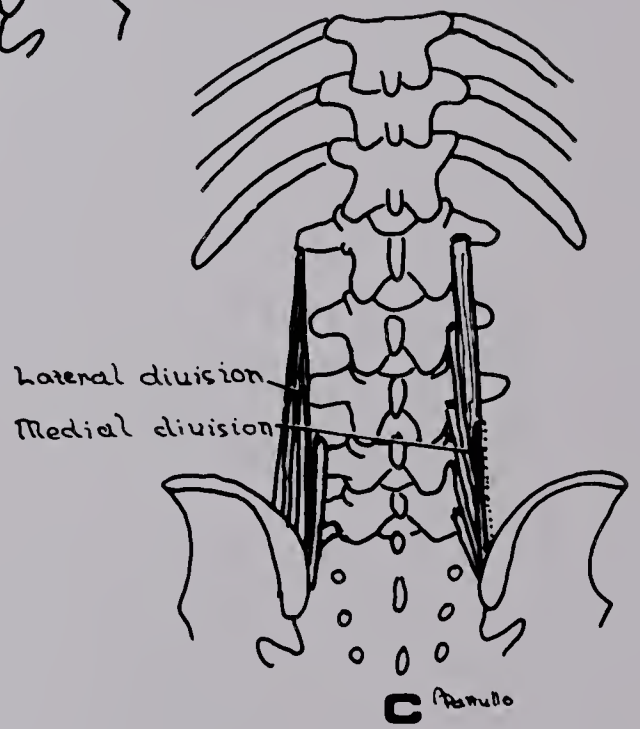
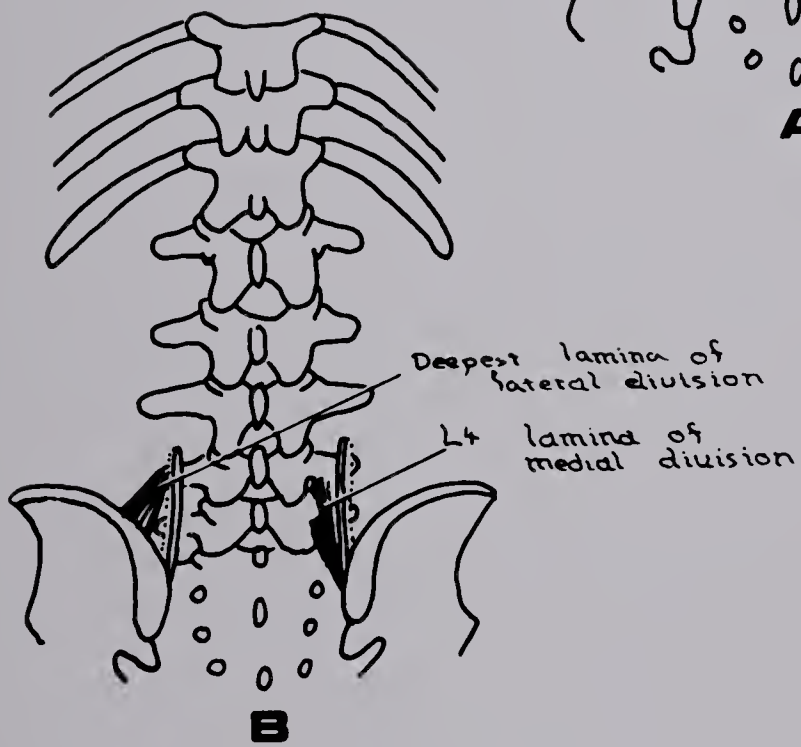
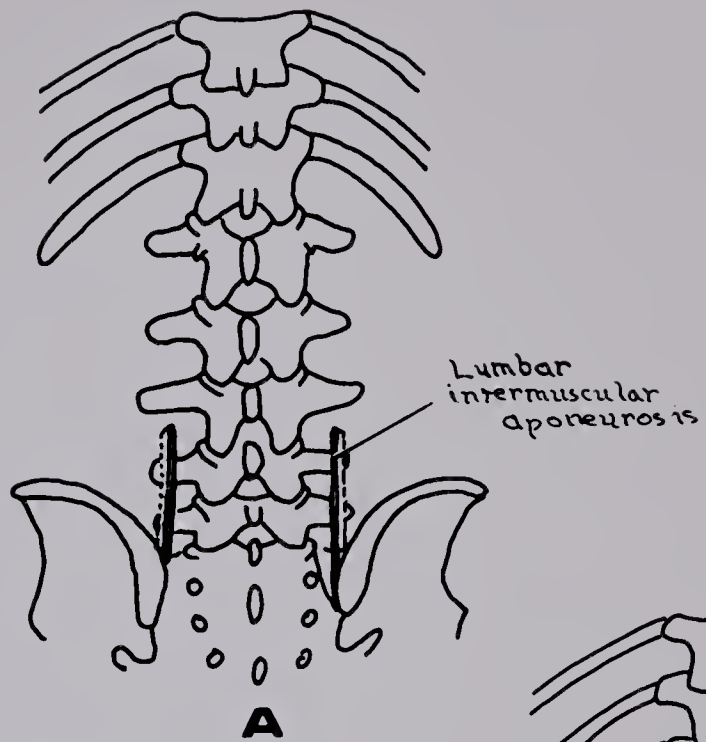


Plate 9: LUMBAR INTERMUSCULAR APONEUROSIS

A. Lumbar intermuscular aponeurosis

B and C. Underlying laminae of the lateral and medial divisions of the erector spinae muscle.

See Appendix A for description.



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